the On & Off of Human Allergies

by Byron Goldstein and Micah Dembo

A study of the interaction between allergen molecules and a cellular surface bristling with antibodies has revealed details about the on-off switches for release of chemicals that cause allergic symptoms.

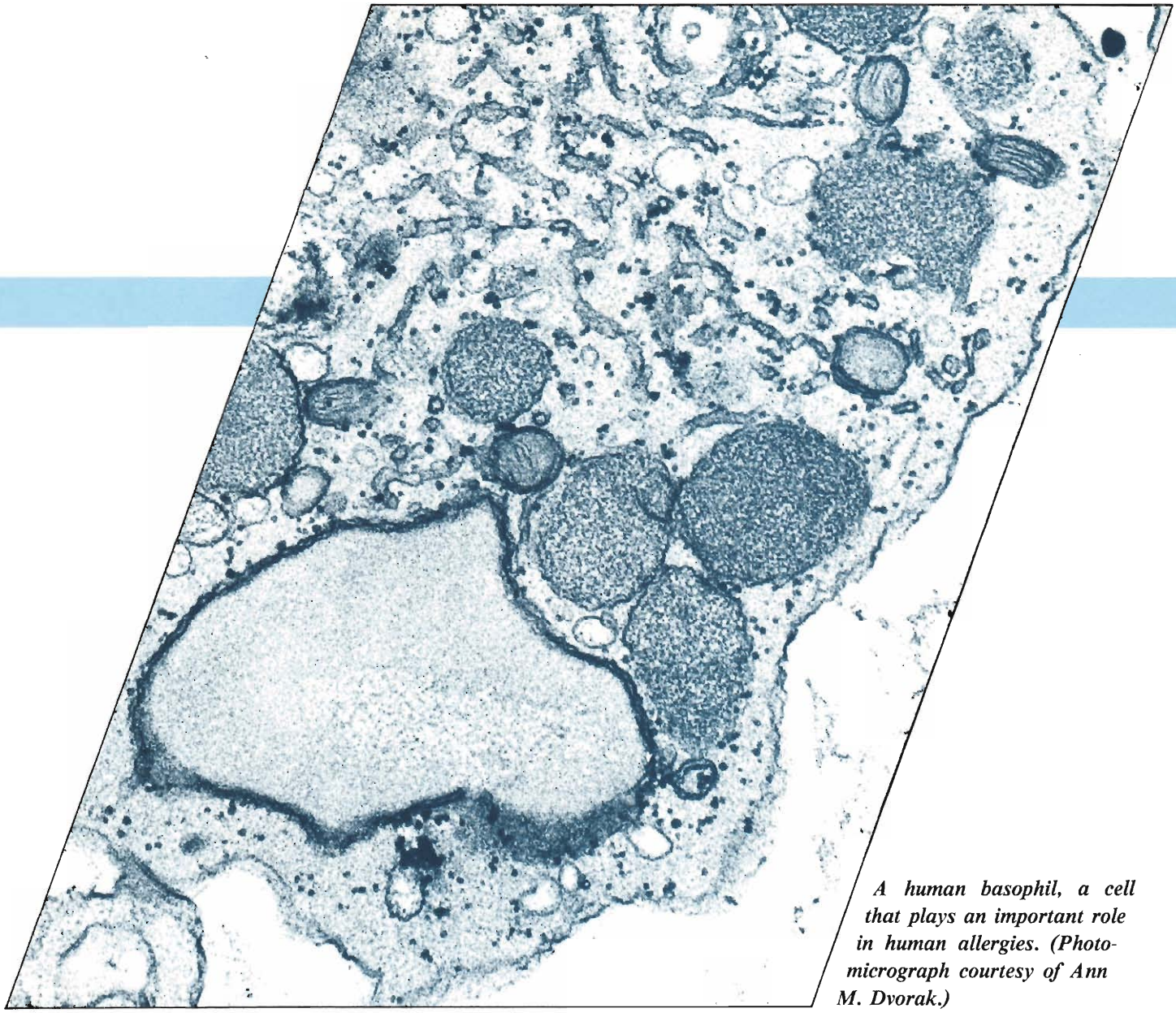
When spring arrives in New Mexico, invisible, diffuse clouds of juniper pollen drift through the air leaving behind a wake of red eyes, runny noses, and sneezing, suffering people. How is it that such a tiny, seemingly harmless substance can wreak such havoc? Why are some people victims, others not? How might this and other allergies be brought under control? Regrettably, allergies are the result of complex biochemical reactions that start in the body's immune system, and answers to our questions require detailed knowledge of these reactions.

The recent explosive growth in immunology has provided some of the knowledge by uncovering many of the steps in allergic reactions. Lawrence Lichtenstein and Anne Kagey-Sobotka at the Johns Hopkins University School of Medicine and we at Los Alamos have been conducting a joint study of a step important in most allergies—the release of histamine, one of the chemicals that cause the allergy sufferer's discomfort. By coupling experimental measurements of histamine release (Johns Hopkins) with theoretical modeling of the pertinent reactions (Los Alamos), it was possible to map this step in detail. The study also provided important information about desensitization, that is, the turning off of the allergic response. It is hoped that this study will bring us closer to control of this common, but often debilitating and sometimes deadly, phenomenon.

The Immune Response

The human immune system is an amazing defense system capable of distinguishing what is foreign from what is self. This system responds to and destroys invading substances that can cause infection or disease, but in some people it also responds to many seemingly harmless foreign substances. Whether it be a virus, a bacterium, or only that troublesome pollen, any foreign material that triggers an immune response is called an antigen.

The immune system reacts to antigens with one or both of two responses, a cellular response and an antibody response. The cellular response involves production of special white blood cells (T lymphocytes) that are capable of binding to and destroying the antigen. A classic example of this response is the rejection of organ transplants. Material from an organ grafted to a genetically different recipient of the same species acts as an antigen and elicits a cellular immune response that destroys the graft.



A human basophil, a cell that plays an important role in human allergies. (Photomicrograph courtesy of Ann M. Dvorak.)

In the second response the immune system reacts to an antigen by producing and releasing into the blood special proteins called antibodies. A popular immunology experiment exemplifies this response. If a mouse is injected with red blood cells from a sheep, the mouse's immune system produces antibodies that can bind to the sheep's red blood cells. The bound antibodies act as tags that clearly advertise the foreign nature of the antigen. Once tagged, the antigen becomes subject to attack and destruction by other molecules and cells of the body.

An important characteristic of the reaction between an antigen and an antibody or T lymphocyte is its specificity: an antibody or a T lymphocyte binds to the antigen that triggered its formation but with rare exceptions does not bind to other antigens. For example, the antibody that binds to influenza A virus does not bind to influenza B virus. As shown in Fig. 1, this specificity occurs because the shape and charge distribution of a particular molecular structure on the antigen—the binding site—are matched by a complementary shape and charge distribution of a binding site on the antibody or T lymphocyte. The two structures mesh somewhat like a lock and key.

Both immune responses possess the property of memory. If a rejected graft is followed by another from the same donor, it will be rejected more rapidly than the first. If a mouse is injected a second time with red blood cells from a sheep, it will produce greater amounts of antibodies more rapidly, and these antibodies will bind more strongly to the sheep's red blood cells.

With these marvelous properties the immune system seems designed to protect us from disease and infection. However, as we have said, not all immune responses are directed against harmful substances and not all immune responses improve our well being, as those of us who have allergies well know.

Allergic Reactions, IgE, and Histamine

There are two types of hypersensitive, or allergic, reactions, each involving one of the two immune responses. Delayed hypersensitive reactions (so called because they evolve slowly, peaking in about 1 to 4 days) involve the cellular, or T lymphocyte, response. Examples are allergies to poison ivy and industrial chemicals. Immediate hypersensitive reactions, on the other hand, involve the antibody response. In the remainder of the article, we will discuss only immediate hypersensitive reactions, and there certainly are enough of them. Hay fever, hives, and asthma, as well as allergies

to grasses, dog and cat danders, certain foods, bee venom, and penicillin, are all examples of antibody-mediated hypersensitivity.

Human antibodies are grouped into five classes (immunoglobulin A, D, E, G, and M) according to their biological functions, and the immune system can produce all these classes in response to normally harmless foreign substances. But no allergic symptoms will result unless immunoglobulin E (IgE) antibodies are produced. An antigen that causes the immune system to produce IgE antibodies is called an allergen.

IgE antibodies were discovered in 1967 by Kimishige Ishizaka and Teruko Ishizaka when they were studying the blood serum of hay-fever patients at the Children's Asthma Research Institute and Hospital in Denver. This class of antibodies was the last to be discovered, in part because it is normally produced in very small amounts. The concentration of all antibodies in the serum of a nonallergic, healthy person is about 15 milligrams per milliliter, but the concentration of IgE antibodies is only about 0.0001 milligrams per milliliter. Allergic individuals, however, tend to have higher concentrations of IgE antibodies. (People suffering from certain parasitic infections also have elevated levels of IgE antibodies, but why this is so is still an open question.)

All antibodies, regardless of class, are made up of similar Y-shaped units. Each unit contains two identical heavy polypeptide chains whose molecular weight depends on the antibody class and varies from 55,000 to 75,000 daltons. These heavy chains are joined by one or more disulfide bonds along some portion of their lengths to form the base of the Y, called the Fc region. The remaining lengths of the heavy chains are free and form flexible arms of the Y. Each arm, or Fab region, also contains a light polypeptide chain joined to the heavy chain by a disulfide bond. The light chains in the two arms are identical and have a molecular weight of about 23,000 daltons. As shown in Fig. 2, IgE antibodies are monomers; that is, they consist of only one of these Y-shaped units. (IgD and IgG are also monomers, IgM is a pentamer, and IgA exists as a monomer, dimer, or trimer.)

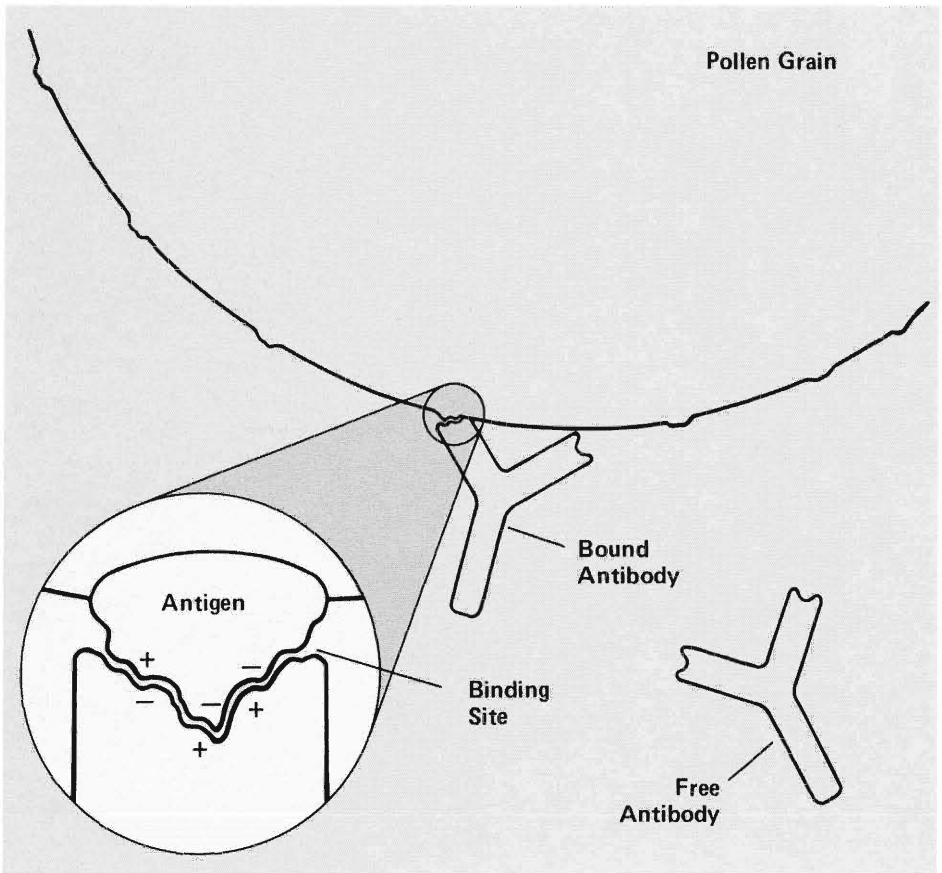


Fig. 1. Antibody-antigen binding. The "lock-and-key" model depicted here explains the high specificity of antibody-antigen binding. The bond between the two is not covalent, but is due to a combination of ionic and induced-dipole dispersion forces. As a result, a strong bond occurs when both the ionic charges and the shapes are complementary, the latter permitting the two surfaces to approach closely and thus maximize the dispersion force. The Y-shaped antibody molecule has two identical binding sites, either or both of which can participate in the binding.

It is the Fc region of an antibody that interacts with cells and molecules of the body and thus determines the antibody's biological functions. A class of antibodies includes all those antibodies with identical Fc regions. The name Fc arises from the fact that these identical fragments can be crystallized from a sample of, say, IgE antibodies. (Crystallization would not occur if these fragments were heterogeneous.)

Identical antigen binding sites are located near the ends of the Fab regions. (The name Fab stands for antigen binding fragment.) Here the antibody's specificity is determined: the binding sites have the correct three-dimensional structure to attach to the sites on the antigen that triggered the antibody's

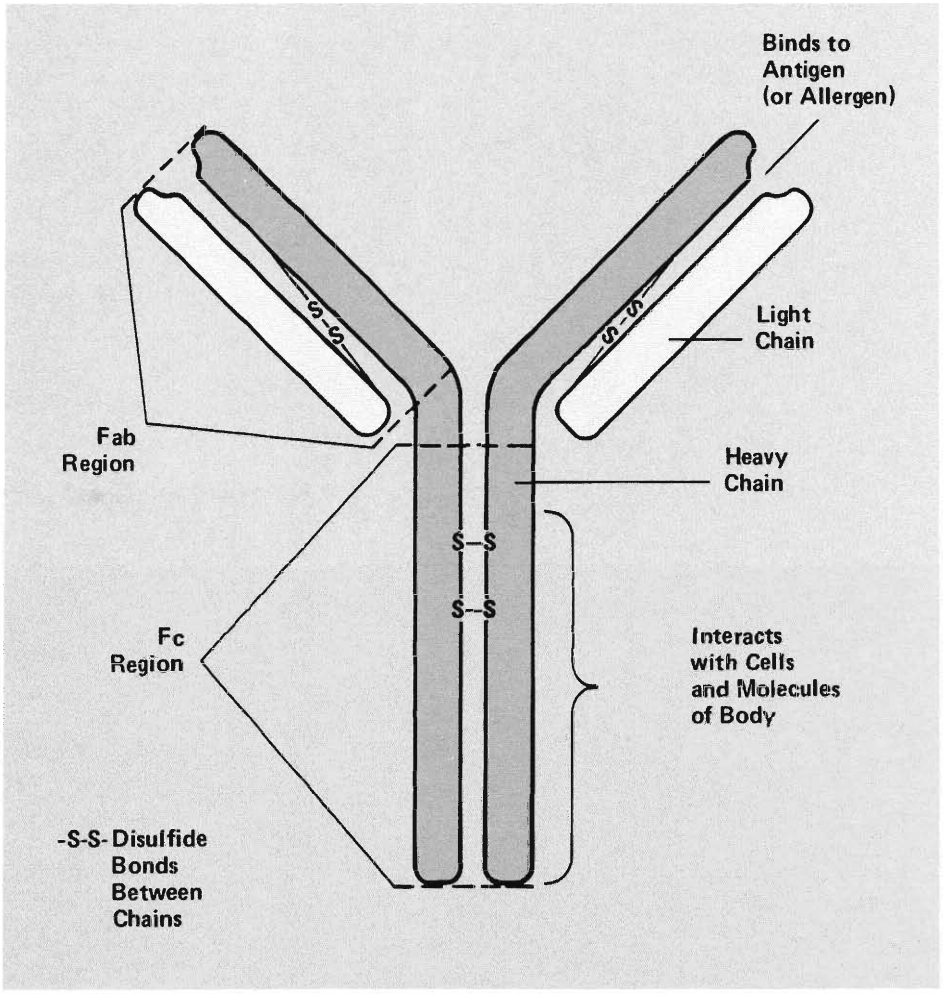


Fig. 2. Important features of an IgE antibody molecule. The molecule consists of two identical heavy polypeptide chains and two identical light polypeptide chains linked by disulfide bonds into an overall Y shape. The configuration depicted here is only symbolic of complicated three-dimensional chains that intertwine and fold back on themselves. Each arm is flexible and the angle between the arms of the Y can vary, possibly from 0 to 180 degrees. The Fc region is identical in all IgE antibodies. This region interacts with the cells and molecules of the body and thus determines the biological functions of the IgE class of antibodies. IgE antibodies specific to different antigens (or allergens) differ in their Fab regions. At the end of each arm are binding sites that match the binding site on the antigen (or allergen) that caused formation of the antibody. The binding sites are here depicted by concave half circles.

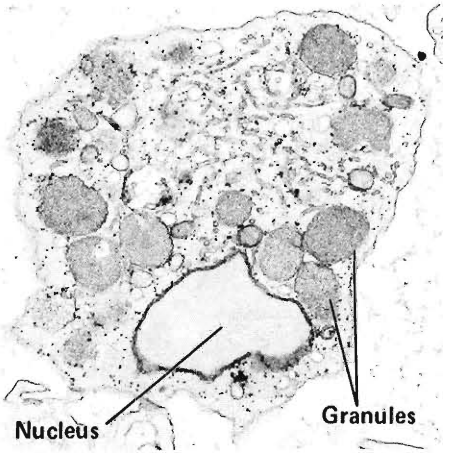


Fig. 3. Photomicrograph of a human basophil (7,000×). During an allergic reaction the granules of these cells release histamine and other potent chemicals into the blood stream. (This photo, kindly supplied by Ann M. Dvorak of the Beth Israel Hospital in Boston, is copyrighted by the United States-Canadian Division of the International Academy of Pathology and is reproduced with their permission.)

production. Thus, antibodies of the same class but specific to different antigens are identical in their Fc regions and differ in their Fab regions.

IgE antibodies interact with two types of cells—basophils and mast cells—that play a central role in allergic reactions. Mast cells are found in tissue and are most numerous in the linings of the respiratory and gastrointestinal tracts and under the skin. Basophils (Fig. 3) are found in blood serum and make up approximately 1 per cent of the white blood cells. The surfaces of both these types of cells are dotted with binding sites for the Fc portion of IgE antibodies. These binding sites are called Fc_ϵ receptors (the epsilon subscript indicates specificity for IgE antibodies). The number of receptors per cell varies widely. For example, on a human basophil there may be anywhere from 5000 to 500,000. Basophils of allergic people tend to have considerably more Fc_ϵ receptors than do those of nonallergic people.

Stored in granules within basophil and mast cells are biologically potent molecules, including histamine and the recently discovered leukotrienes. When these molecules are released from the cells they have several important effects. They cause contraction of smooth muscles such as those surrounding blood vessels and air passages in the lungs. It has been shown that histamine contracts the smooth muscles of the larger air passages in the lungs, and the leukotrienes contract the smaller peripheral airways. These molecules also affect the permeability of blood vessel walls and other membranes and cause glandular hypersecretion. At the site of a puncture wound, these effects result in blood flow changes, inflammation, fluid secretion, and the passage through various membranes of molecules and cells that attack and destroy harmful substances. In an allergic reaction a body-wide release of these same chemicals results in symptoms such as fluid secretion in the nose and throat and obstruction of air passages in the lungs.

There are several steps leading up to

histamine release; these are depicted schematically in Fig. 4. The first step is the initial response of a person's immune system to an allergen: the production of IgE antibodies specific to the allergen. Some of these IgE antibodies then bind through their Fc regions to Fc_ϵ receptors on mast cells or basophils; the two arms of the Y and the allergen-specific binding sites project outward from the cell surface. The cells are now "sensitized" to the allergen. Another exposure to the same allergen results in binding between the allergen molecules and the IgE antibodies that are bound to the surfaces of the cells. It is this interaction that

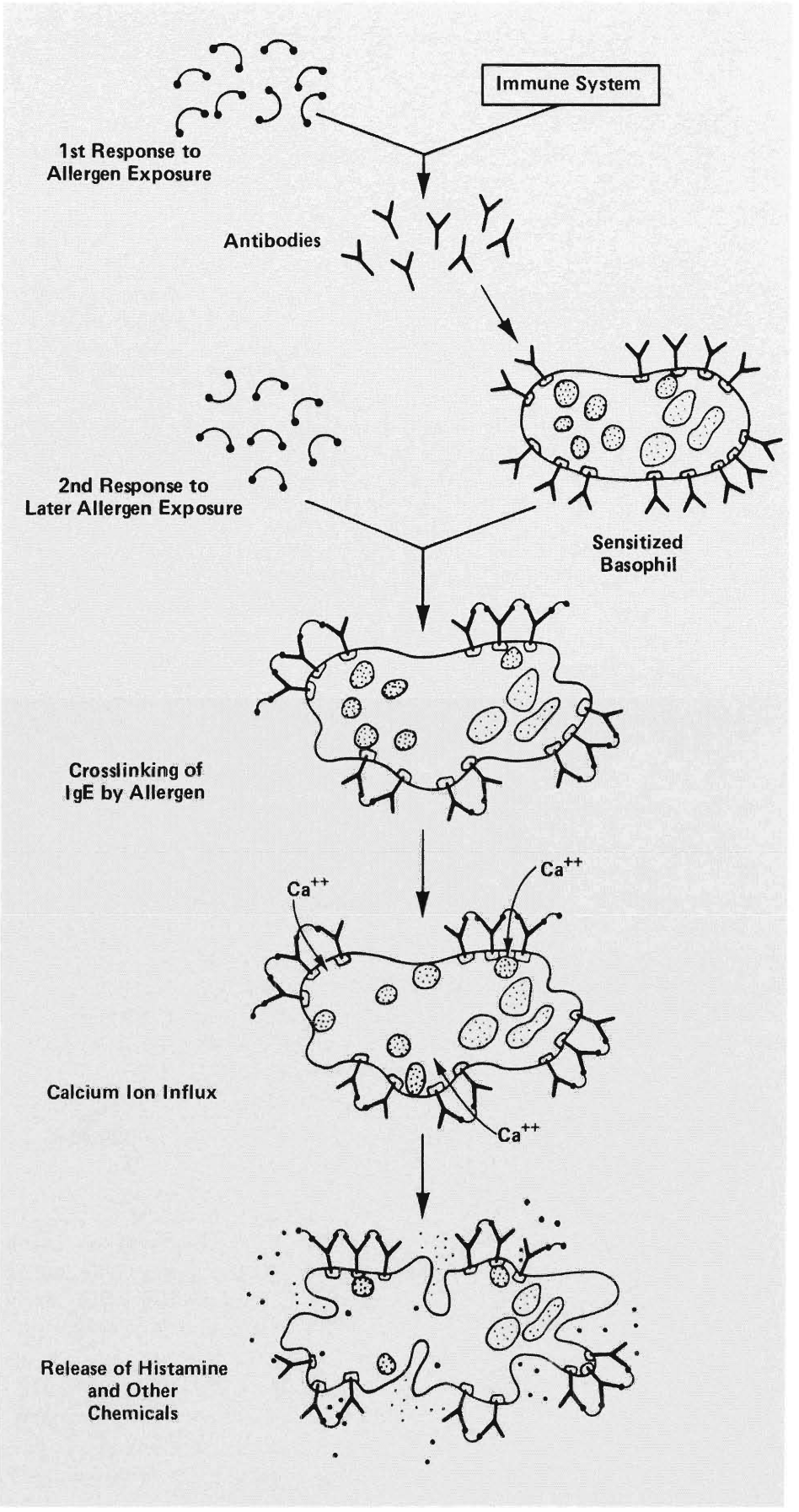


Fig. 4. The allergic reaction. The body's immune system may react to an allergen by producing IgE antibodies specific to that allergen. Many of these antibodies bind to the surfaces of basophils and mast cells through receptors that are specific to the Fc region of IgE antibodies; the arms of the antibodies containing the binding sites specific to the allergen point outward. A cell stippled with antibodies specific to an allergen is said to be sensitized to that allergen. Another exposure to the same allergen can lead to the formation of crosslinks, or bridges, between the IgE antibodies bound to the cell surfaces. These crosslinks form as sites on an allergen bind to complementary sites on adjacent antibodies. (Note that the allergen must have more than one binding site to form crosslinks. For simplicity the allergen shown here is divalent; that is, it has two binding sites.) Wherever a crosslink is formed a channel opens for transport of calcium ions (Ca^{++}) into the cell interior. This influx triggers a mechanism in which the granules release their contents of histamine and other chemicals into the blood serum.

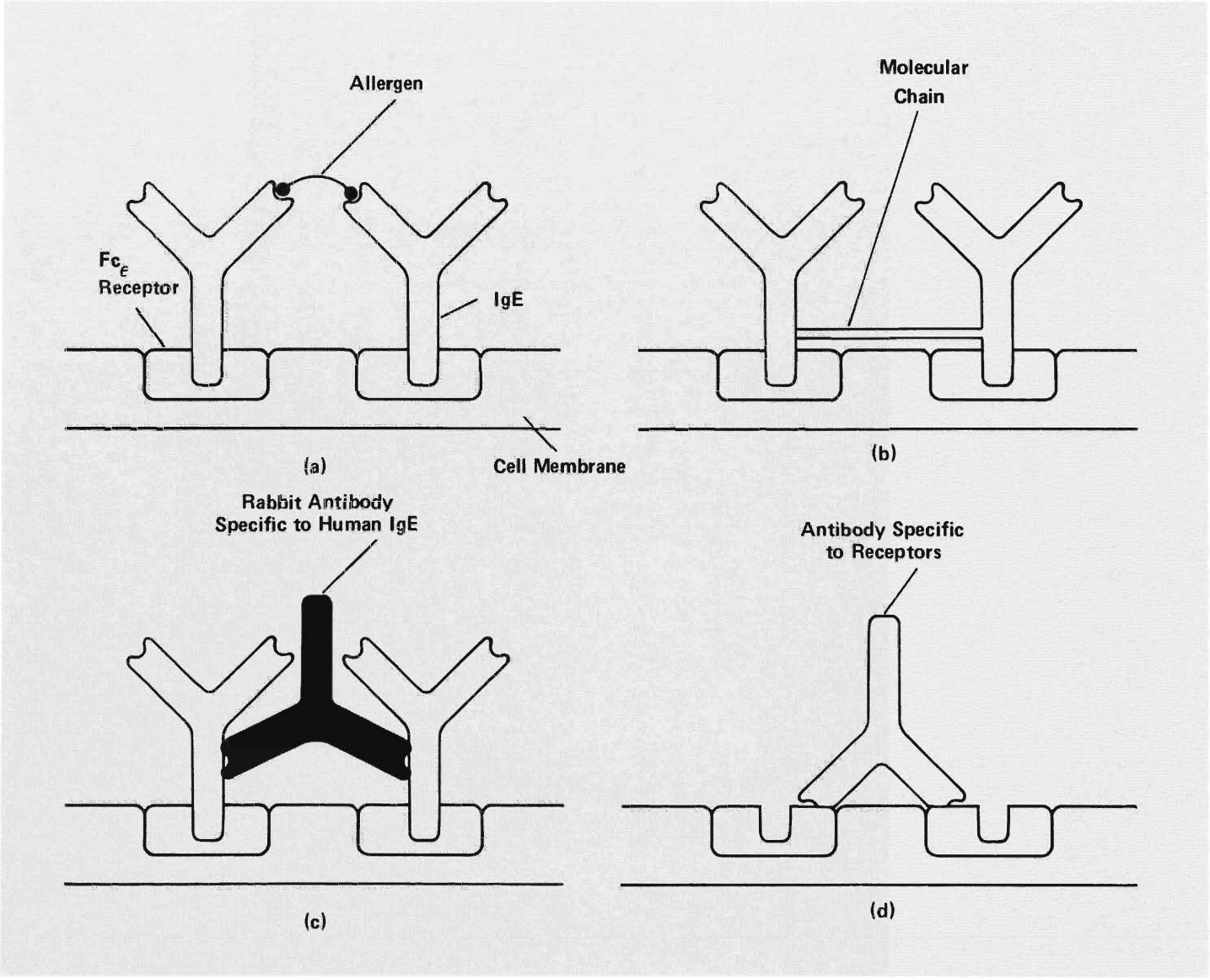


Fig. 5. A necessary condition for the release of histamine from a basophil is that two or more Fc_ϵ receptors on the cell surface be brought and held in proximity. This condition is achieved as any of the following processes takes place: (a) allergens form crosslinks between the arms of IgE antibodies bound to the receptors; (b) a molecular chain covalently binds two IgE antibodies bound to the receptors; (c) a rabbit antibody specific to human IgE antibodies binds to the Fc region of IgE antibodies bound to the receptors; and (d) a specially prepared antibody binds directly to the Fc_ϵ receptors.

can trigger the release of histamine from the granules of the cells. Here, then, is a critical step in an immediate hypersensitive allergic reaction.

In the remainder of the article we will discuss the histamine release mechanism in more detail, in particular what turns it on and what turns it off. We will concentrate on basophils, but almost everything we say about those cells holds true for mast cells as well.

The Basophil's "On" Signal

One condition necessary for histamine release from basophils is that the allergens must bind to the IgE antibodies in such a way that crosslinks, or bridges, are formed between the antibodies (Fig. 5a). Evidence that this crosslinking is necessary lies in the fact that allergens with only one binding site, which are physically incapable of crosslinking two IgE antibodies, do not trigger histamine release. Chains, rings, or other configurations of many crosslinked IgE antibodies may form on the basophil surfaces, but such large aggregates of crosslinked antibodies are not necessary for histamine release. Rather, the formation of crosslinked antibody pairs is sufficient. David Segal, Joel Taurog, and Henry Metzger of the National Institutes of Health demonstrated this sufficiency in 1978 by exposing basophils to molecules consisting of two covalently linked IgE antibodies (Fig. 5b). These permanently linked IgE antibody pairs caused histamine release in the absence of allergen.

Allergens crosslink IgE antibodies by binding to their Fab regions, but other molecules that crosslink IgE antibodies by binding along the Fc regions in the base of the Y can also cause histamine release. This phenomenon was originally demonstrated with molecules prepared by injecting rabbits with human IgE antibodies; the rabbits produced antibodies that bound specifically to the Fc regions of human IgE antibodies. (Other animal antibodies specific to human IgE antibodies are prepared in a similar

fashion.) When basophils sensitized with human IgE antibodies were exposed to these rabbit antibodies, histamine release occurred even though the antibodies were crosslinked through their Fc regions (Fig. 5c).

It is now clear that the requirement for histamine release of crosslinked IgE antibodies is really a requirement that Fc_ϵ receptors, which are mobile on basophil and mast cell surfaces, be brought and held in proximity. This was verified in 1978 by the Ishizakas and their collaborators at Johns Hopkins University, as well as a group at the National Institutes of Health, when they made an antibody that bound to Fc_ϵ receptors on mast cells and basophils of rats. This antibody triggered histamine release from the cells simply by bringing empty Fc_ϵ receptors close together (Fig. 5d).

Histamine release from basophils also demands another condition. If no calcium ions are present in the medium surrounding the basophils, no histamine will be released. The proximity of Fc_ϵ receptors brought about by crosslinked IgE antibodies somehow allows calcium ions to cross the cell membrane, and this influx of calcium ions is an essential signal for histamine release. If calcium ions can be introduced into the cell in some other way, crosslinked IgE antibodies are not needed. For example, calcium ionophores, substances that cause calcium ion channels to form in cell membranes, will induce histamine release in the absence of crosslinking if calcium ions are available. Injecting calcium ions directly into basophils also induces histamine release. In a test tube the calcium ion concentration can be manipulated, but in serum, the natural milieu of the basophil, calcium ions are always present at a concentration (2 to 5 millimolar) that is sufficient to insure histamine release.

In summary, an immediate hypersensitive reaction is turned on by the flow of calcium ions into sensitized basophils made possible by allergen-linked IgE antibodies on the basophil surfaces. What turns the reaction off?

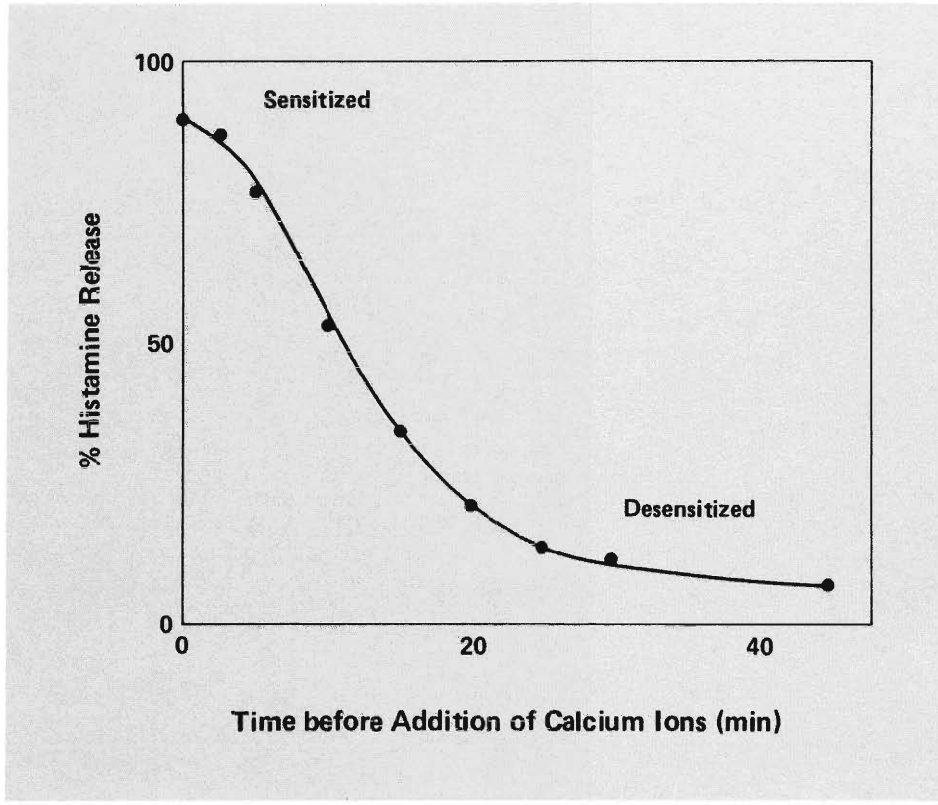


Fig. 6. Basophil desensitization. Basophils sensitized to the major ragweed allergen release a large percentage of their histamine when exposed to the allergen in the presence of calcium ions. (The basophils studied in the experiment depicted here released almost 90 per cent of their histamine under these circumstances.) However, if the basophils are exposed to the allergen in the absence of calcium ions, the cells gradually lose their ability to release histamine. The cells are said to be desensitized when very little histamine is released in the presence of calcium ions.

A Nonspecific "Off" Signal

As early as 1964 Lawrence Lichtenstein and Abraham Osler at Johns Hopkins University showed that the same agent that caused histamine release from basophils of hay-fever patients, ragweed pollen, could also desensitize these cells, that is, block their release of histamine. In 1971 Lichtenstein investigated desensitization further with experiments on white blood cells, including basophils, of hay-fever patients. The cells were exposed for various lengths of time to the major ragweed allergen (a 38,000-dalton protein isolated by T. P. King and his collaborators at Rockefeller University) in a medium containing no calcium ions. Calcium ions were then added, and the amount of histamine released by the cells during the following 30 minutes was measured. These experiments showed that the longer the cells were exposed to the ragweed allergen in the absence of calcium, the less histamine they were capable of releasing in the presence of calcium (Fig. 6). Lichtenstein obtained similar results using as the crosslinking agent

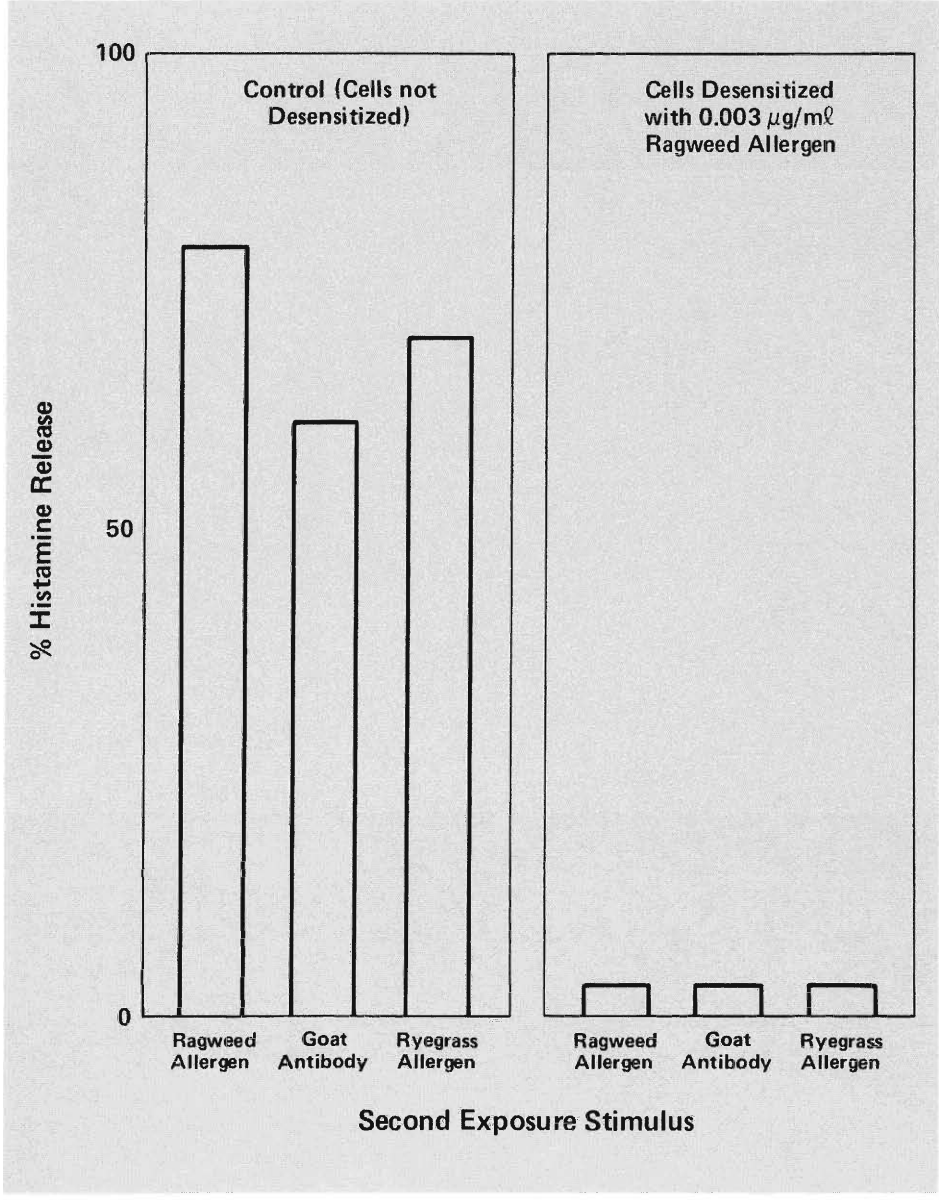


Fig. 7. Nonspecific desensitization. The basophils studied in the experiment illustrated here were from a donor who was allergic to both the major ragweed allergen and a ryegrass allergen. On the left are the percentages of histamine released by these doubly sensitized basophils when exposed to either of the two allergens or to a goat antibody specific to human IgE antibodies. On the right are the percentages of histamine released by these cells when exposed to the ragweed or ryegrass allergens or the goat antibody after *being desensitized to the ragweed allergen. The negligible release shows the cells to be nonspecifically desensitized to any allergen or crosslinking agent.*

a rabbit antibody specific to human IgE antibodies. It appears that the crosslinking of IgE antibodies on the basophil surface initiates two signals: a signal for histamine release in the presence of calcium ions and a signal for blockage of histamine release in the absence of calcium ions.

Lichtenstein performed similar experiments on white blood cells from a donor who was allergic to both the ragweed allergen and a ryegrass allergen. Therefore, some of the Fc_ϵ receptors on the donor's basophils were occupied by IgE antibodies specific to the ragweed allergen and some by IgE antibodies specific to the ryegrass allergen. As before, after sufficient exposure to the ragweed allergen in the absence of calcium ions, addition of calcium ions caused no significant histamine release. The cells, now desensitized to the ragweed allergen, were exposed in the presence of calcium ions either to the ryegrass allergen or to a goat antibody specific to human IgE antibodies. Neither substance caused significant release (Fig. 7). Desensitization to one allergen turned the basophils off to other allergens and crosslinking agents as well. This "nonspecific" desensitization occurred despite the fact that the desensitizing allergen interacted with only a fraction of the IgE antibodies on the cell surfaces, namely, those specific to the ragweed allergen.

Although nonspecifically desensitized basophils cannot be made to release histamine by crosslinked IgE antibodies, they do release histamine when exposed to calcium ionophores or injected with calcium ions. Nonspecific desensitization must therefore involve the shutdown of a calcium ion transport mechanism. Apparently, crosslinking of IgE antibodies in the presence of calcium ions at first activates the transport mechanism, but with time that activation somehow degrades.

Blocking histamine release by withholding calcium ions represents an artificial situation. Does nonspecific desensitization occur in the natural milieu of the basophil, that is, in the

presence of calcium ions? Evidence for a positive answer comes from numerous *in vitro* studies of histamine release from basophils as a function of allergen concentration. Data from such studies are generally displayed as plots, known as dose response curves, of the percentage of histamine released versus the logarithm of the allergen concentration. Figure 8 shows dose response curves for basophils from three allergic donors. The initial rise of the dose response curves reflects the increase in the number of IgE antibodies that are crosslinked by the allergen.The more interesting feature of the curves is their eventual fall, a phenomenon known as allergen excess inhibition.

Karl Becker, Henry Metzger, and Philip Grimley of the National Institutes of Health, in collaboration with the Ishizakas, showed that for basophils from allergic donors, which generally have large numbers of specific IgE antibodies on their surfaces, excess inhibition is accompanied by large numbers of crosslinks. They did so by studying the distribution of IgE antibodies crosslinked by a fluorescent form of a sheep antibody specific to human IgE antibodies (Fig. 9). At low concentrations of the sheep antibody the distribution of fluorescence was diffuse, but, at the concentrations at which excess inhibition occurs, the distribution of fluorescence became patchy. These observations suggest that at high concentrations of the crosslinking agent large aggregates of crosslinked IgE antibodies had formed.

Excess inhibition can be understood in terms of the effect of crosslinking on the calcium ion transport mechanism. Low numbers of crosslinks (low allergen concentrations) activate the transport mechanism and histamine release occurs. Degradation of the transport mechanism takes place slowly, and histamine release can be blocked only by withholding calcium ions until this gradual shutdown has been completed. As the number of crosslinks increases (higher allergen concentrations), the transport mechanism degrades more rapidly. Eventually, degradation dominates and histamine release is

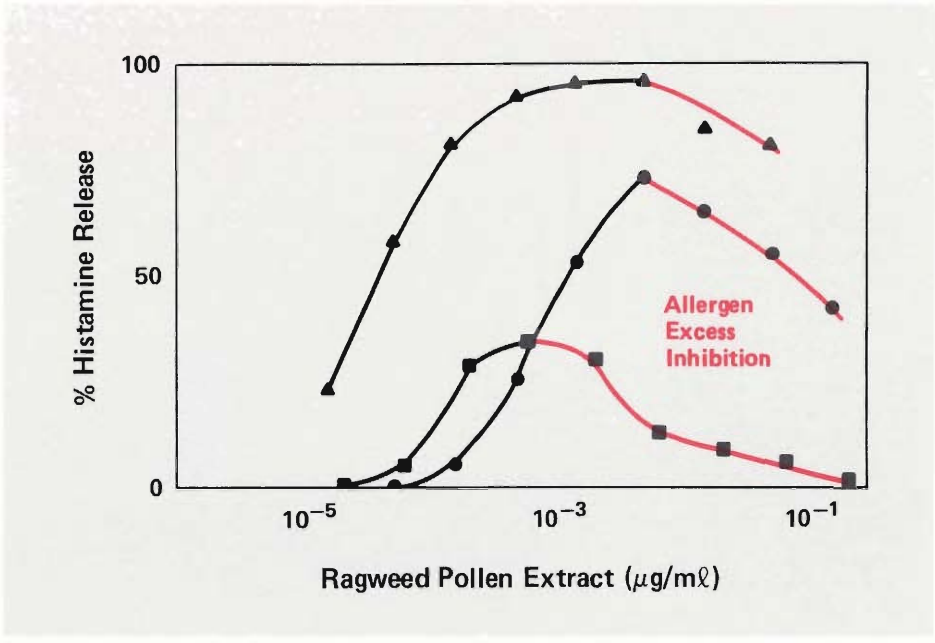


Fig. 8. Typical dose response curves. These curves show the percentages of histamine released by basophils from three ragweed-pollen-allergic donors when exposed to various concentrations of ragweed pollen extract. In all cases the percentage of histamine released first rises with increasing concentration but then peaks and declines. This decline at high allergen concentrations is called allergen excess inhibition. The ragweed pollen extract used in the experiment contained five ragweed allergens. From Lawrence M. Lichtenstein and Abraham G. Osler, Journal of Experimental Medicine 120, *507 (1964).*

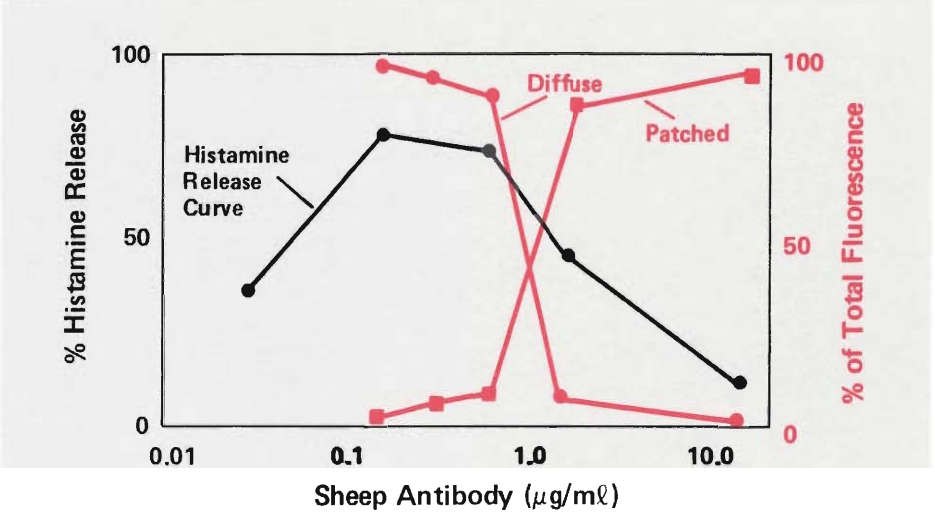


Fig. 9. At low concentrations of a sheep antibody specific to human IgE antibodies, the dose response curve (black) is rising and, as shown by fluorescence microscopy (red), the distribution of IgE antibodies on the basophil surface is mainly diffuse. At concentrations above that for maximum release of histamine, the distribution of IgE antibodies becomes mainly patched. The patches of fluorescence indicate the formation of large aggregates of crosslinked antibodies. Based on data from Karl E. Becker, T. Ishizaka, H. Metzger, K. Ishizaka, and Philip M. Grimley, Journal of Experimental Medicine 138, *394 (1973).*

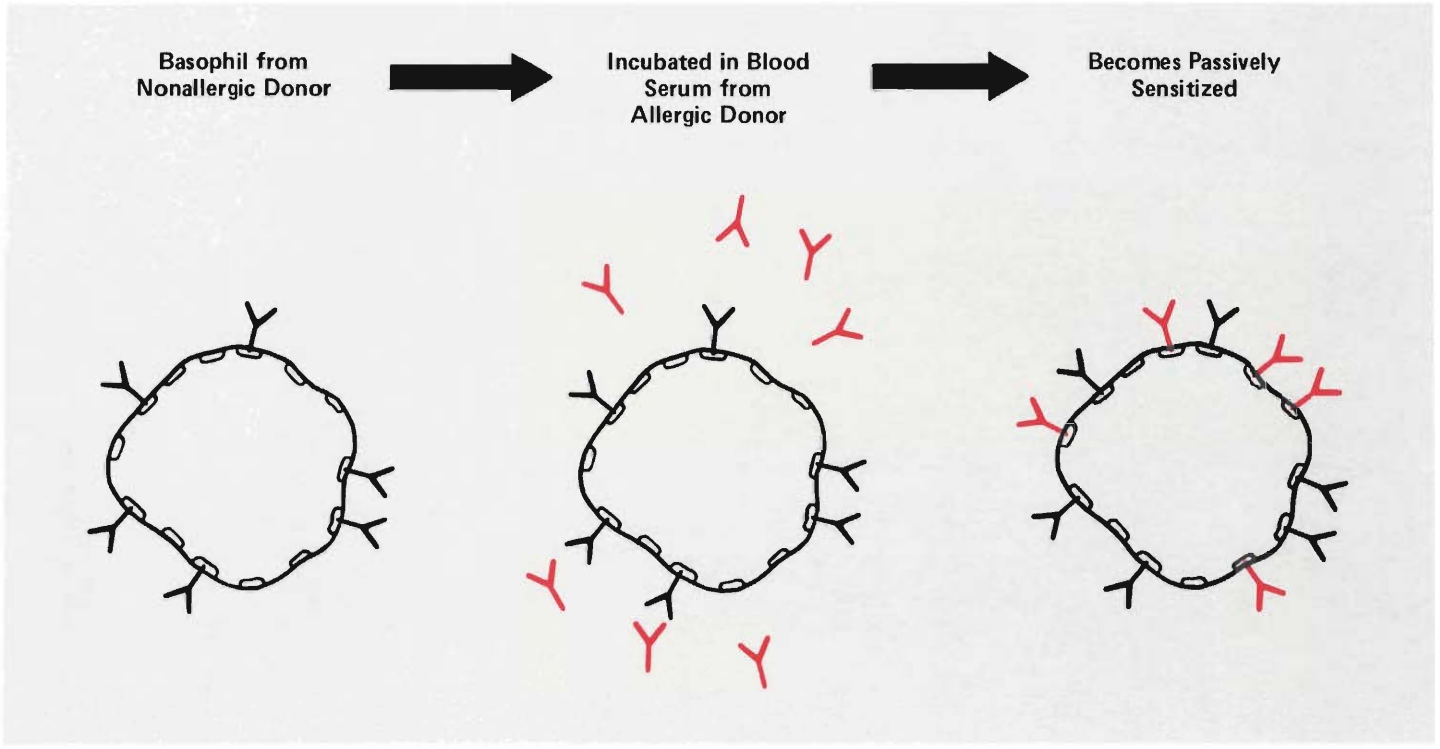


Fig. 10. Passive sensitization. Basophils with empty Fc_ϵ receptors can be sensitized to an allergen by incubating the cells in blood serum from a donor who is allergic to the allergen. This passive sensitization occurs because IgE antibodies specific to the allergen (red) fill some of the empty Fc_ϵ receptors.

blocked even in the presence of calcium ions.

Only on basophils with large numbers of the allergen-specific IgE antibodies bound to their surfaces can there be sufficient crosslinks for nonspecific desensitization to occur in the presence of calcium ions. Therefore, generally only basophils from allergic donors exhibit allergen excess inhibition because a substantial fraction of their large number of Fc_ϵ receptors is filled with the allergen-specific IgE antibodies.

What happens, however, when there are only small numbers of the allergen-specific IgE antibodies on the basophil surfaces? The answer to this question was provided by our joint research with Lichtenstein and Kagey-Sobotka. What happens proved to be not only something different but also quite interesting. In particular, we observed another mechanism for turning off the allergic reaction.

The Experiments

The goal of our initial collaboration with Lichtenstein and Kagey-Sobotka in 1977 was to test our theoretical predictions, in particular, predictions we had made about the dose response curves as the number of allergen-specific IgE antibodies on the basophil surfaces is increased. To eliminate extraneous variables that would blur a correlation between theory and experiment, we needed to use the same allergen, the same allergen-specific IgE antibody, and basophils from the same donor. Moreover, we needed basophils with free Fc_ϵ receptors that could be filled with different amounts of the IgE antibody of our choice. This requirement forced us to use basophils from nonallergic donors, because basophils from allergic donors tend to have most of their Fc_ϵ receptors filled. However, the total number of receptors is much smaller on basophils from nonallergic donors. As a result, our study was of basophils with relatively small numbers of the allergen-specific IgE antibodies on their surfaces, usually less than 10,000 per cell. Serendipitously, this circumstance led to our discovery of a second mechanism for desensitization.

Other experimental restraints resulted from the fact that no one had, or has yet, learned how to keep human basophils alive outside the body for longer than a day or so. The research thus depended on the availability of donors, both of basophils and blood serum. Another difficulty was the day-to-day variation in the basophil donor's exposure and immune system response that changed the number of empty Fc_ϵ receptors on the basophil surfaces.

To obtain basophils with different and well-characterized numbers of the allergen-specific IgE antibody on their surfaces, we incubated the cells with free Fc_ϵ receptors in serum from a donor who was extremely allergic to penicillin. (Ninety per cent of the penicillin-allergic donor's IgE antibodies were specific to the benzylpenicilloyl, or BPO, group.) The length of the incubation or the dilution of the penicillin-allergic donor's serum determined how many BPO-specific IgE antibodies filled Fc_ϵ receptors on the basophil surfaces.

This technique for sensitizing cells is called passive sensitization (Fig. 10) and has been known since 1921 when Carl Prausnitz injected into the skin of a nonallergic subject a small quantity of serum from Heinz Küstner, who was extremely allergic to fish. Twenty-four hours later fish extract was injected into the same area of the nonallergic subject's skin. Immediately a wheal appeared. We now know that Küstner's serum contained IgE antibodies specific to an allergen found in fish. When transferred to the skin of the nonallergic subject, these antibodies passively sensitized his mast cells. Then, when exposed to the allergen in the fish extract, the sensitized mast cells released

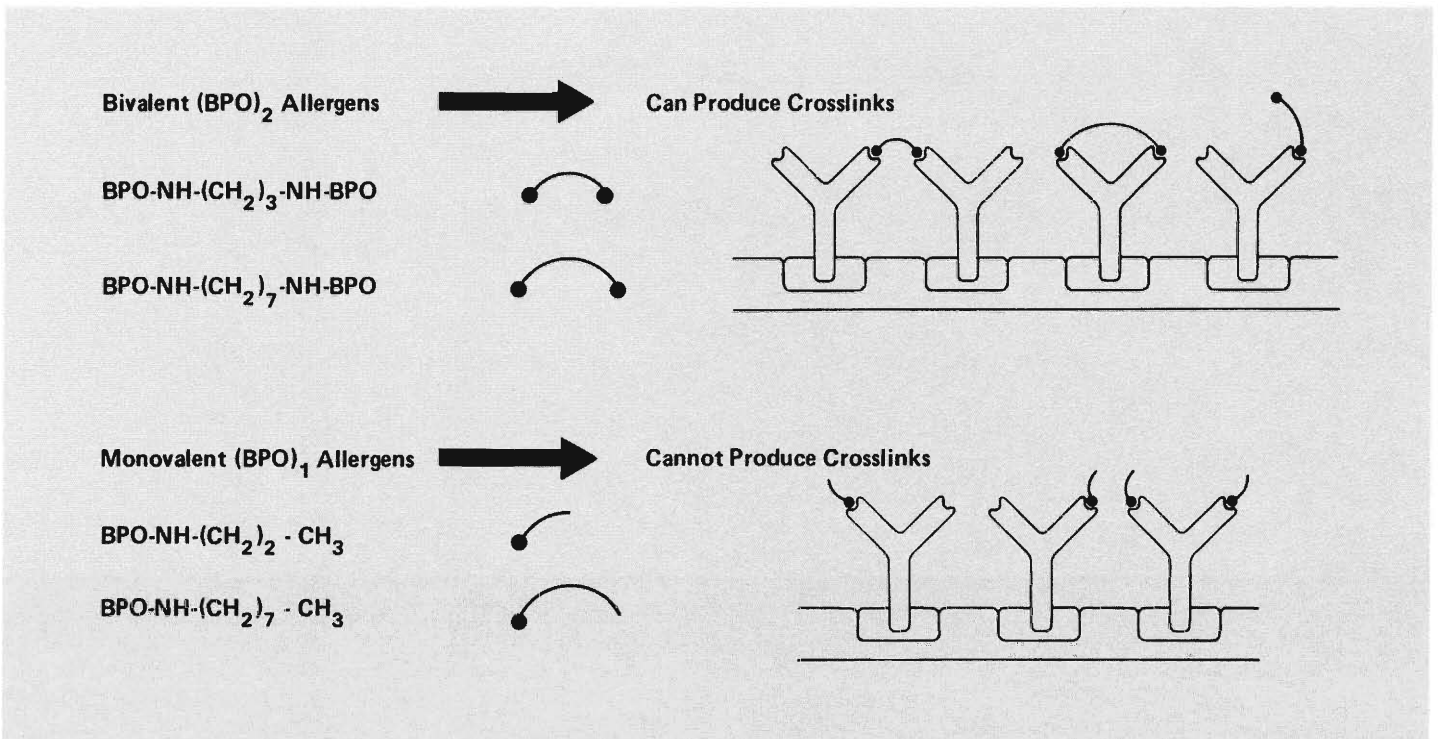


Fig. 11. Synthetic benzylpenicilloyl (BPO) allergens. The bivalent molecules, with a BPO group (circle) at each end of the chain, are able to form bridges between binding sites on the arms of the IgE antibodies. The monovalent molecules, with only one BPO group, cannot form such crosslinks.

histamine and other chemicals that produced the wheal. The PK test for allergy is named for these early investigators.

Our choice of using IgE antibodies from a penicillin-allergic person was based on the availability of simple, well-defined synthetic penicillin allergens. Bernard Levine at the New York University School of Medicine had first prepared a series of such allergens in 1967. Some of these are illustrated in Fig. 11. Each is a linear chain of different length with either a BPO group at each end (bivalent) or a BPO group at one end only (monovalent).

We resurrected the bivalent and monovalent synthetic penicillin allergens because they were ideal for our purpose: to start to build a mathematical model of histamine release from basophils. In $(BPO)_2$ we had the simplest possible crosslinking agent, a symmetric linear molecule with two identical binding sites. In $(BPO)_1$ we had a tool for testing our ideas about what happens when the number of crosslinks is reduced.

The final step of the experiments entailed determining the dose response curves for the passively sensitized basophils, that is, measuring the percentages of histamine released by the basophils when exposed to various concentrations of $(BPO)_2$ and $(BPO)_1$ allergens. We hoped that by comparing the dose response curves with our theoretical calculations about crosslinks we could learn something new about the role of crosslinks in histamine release.

We shall first present the results of our calculations and then a comparison of these results with those of the experiments.

Results of Crosslinking Calculations

We learned experimentally that the binding between the IgE antibodies used to passively sensitize the basophils and the $(BPO)_2$ or $(BPO)_1$ allergens came to equilibrium within seconds after the basophils were exposed to the allergens and well before any measurable histamine release or desensitization had occurred. Thus, histamine release and desensitization were governed by the equilibrium concentration of crosslinks. For these experiments we could neglect the details of the binding during the first few seconds of exposure and instead treat the basophils as if the crosslinks formed instantaneously. Thus, we calculated, for a given number of BPO-specific IgE antibodies per basophil, the equilibrium concentration of

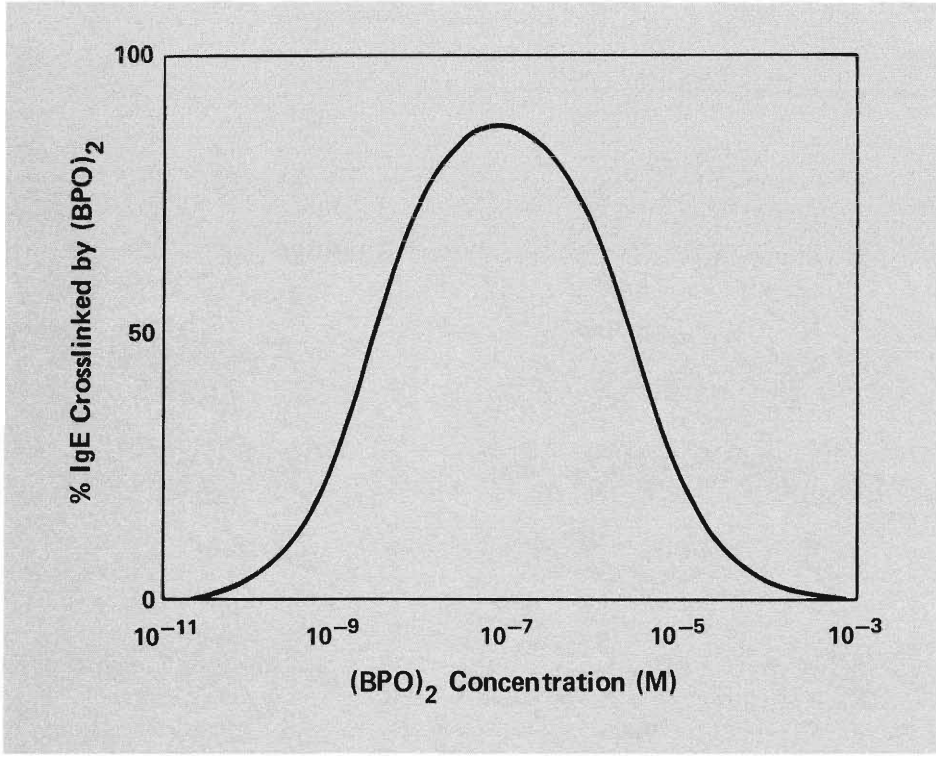


Fig. 12. According to our calculations, the fraction of IgE antibodies crosslinked by $(BPO)_2$ varies as shown with the concentration of $(BPO)_2$ to which the basophils are exposed. The curve has a single maximum and is symmetric about that maximum. The initial rise in the curve is expected; the decline is due to a saturation effect depicted in Fig. 13.

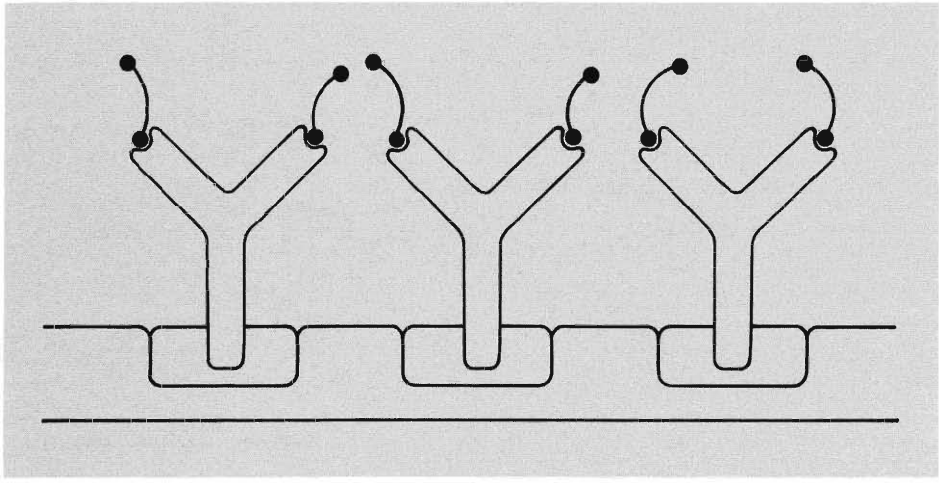


Fig. 13. At high $(BPO)_2$ concentrations most of the binding sites on the antibodies are filled with $(BPO)_2$ molecules. Because there are few empty sites available, these bivalent $(BPO)_2$ molecules are unable to form bridges between the antibodies.

crosslinks on the basophil surfaces for various concentrations of $(BPO)_2$ and $(BPO)_1$ allergens.

Our method of attacking this equilibrium problem is described in the sidebar "Crosslinking—A Theoretical Approach." The results of the calculations revealed interesting predictions about crosslinking. For example, Fig. 12 shows a plot, called a crosslinking curve, of the fraction of crosslinked antibodies versus the logarithm of the $(BPO)_2$ concentration. The curve rises from zero to a maximum and then decreases to zero. The curve falls because, as the $(BPO)_2$ concentration increases, more and more of the binding sites on the antibodies are occupied by $(BPO)_2$ allergens with one unbound BPO group (Fig. 13). With fewer potential sites available for that unbound BPO group the number of crosslinks decreases.

Another property of the crosslinking curves is symmetry about their maxima. That is, if the maximum number of crosslinks occurs at a $(BPO)_2$ concentration of 10^{-7} molar (as it does in Fig. 12), there will be just as many IgE antibodies crosslinked at 10^{-6} molar as at 10^{-8} molar.

The $(BPO)_2$ concentration at which the maximum of the crosslinking curve occurs is determined by only two parameters: K, the equilibrium constant for the binding between a BPO group and a binding site on an antibody, and the concentration of $(BPO)_1$ allergen. If $[(BPO)_2]_{max}$ is the $(BPO)_2$ concentration at which crosslinks are a maximum and $[(BPO)_1]$ is the $(BPO)_1$ concentration to which the basophils are exposed, then

$$[(BPO)_2]_{max} = \frac{1}{2}\left(\frac{1}{K} + [(BPO)_1]\right) \quad . \quad (1)$$

One of the most interesting predictions of our calculations is that $[(BPO)_2]_{max}$ does not depend on the total number of BPO-specific antibodies on the basophil surfaces. In other words, if the number of BPO-specific anti-

Sidebar

crosslinking — a theoretical approach

The problem at hand was to calculate the equilibrium concentration of crosslinks formed when BPO-specific IgE antibodies on basophil surfaces are exposed to the monovalent and bivalent synthetic penicillin allergens $(BPO)_1$ and $(BPO)_2$. We began by constructing a model consisting of all the binding reactions that can occur in this situation. Crucial to the calculations is knowledge of the equilibrium constants for these binding reactions. Although the model includes an infinite number of reactions, some reasonable assumptions reduce to a manageable number the equilibrium constants that must be known.

First, we let K be the equilibrium constant for the binding between a monovalent allergen and a "monovalent antibody." (A monovalent antibody does not, of course, exist but is useful as a theoretical construct because the equilibrium constant for its reaction with a monovalent allergen is indicative of the basic strength of the forces between the binding sites.) Consider now the simplest of the reactions depicted in the accompanying figure, those initial reactions in which a $(BPO)_1$ or $(BPO)_2$ allergen binds to one of the two sites on a free antibody (that is, an antibody bound through its Fc region to the cell surface but with each of the binding sites in its Fab regions free). The equilibrium constant for the reaction when $(BPO)_1$ is involved is $2K$ since the antibody offers two possible binding sites. Similarly, the equilibrium constant for the reaction involving $(BPO)_2$ is simply $4K$ because in this case the allergen also offers two possible binding sites. We assume that the equilibrium constants for these two reactions are unaffected if the free antibody is replaced by a chain of crosslinked antibodies with a free binding site on the antibody at each end of the chain.

Considering next the binding of a $(BPO)_1$ or $(BPO)_2$ allergen to the single available site on the products of the initial reactions, we assume that the equilibrium constants for these reactions are also related to K by appropriate statistical factors.

Next, we let K_x be the equilibrium constant for the basic crosslinking reaction, the binding of the complex containing one antibody and one $(BPO)_2$ allergen, each with a free binding site, to a free antibody. Again we assume that the equilibrium constant is unaffected if the free antibody is replaced by a chain of crosslinked antibodies with a free binding site on the antibody at each end.

Finally, a ring containing i antibodies is formed when a free BPO group of a $(BPO)_2$ allergen bound to one end of a chain of i crosslinked antibodies binds to the free site on the antibody at the other end of the chain. We assume that for $i \geq 2$ the equilibrium constant J_i for such a reaction is inversely proportional to i^2. Therefore, $J_i = 4J_2/i^2$ for $i \geq 2$. We let J_1 be the equilibrium constant for formation of the "ring" consisting of a single $(BPO)_2$ allergen spanning the sites on a single antibody.

Armed with the four equilibrium constants K, K_x, J_1, and J_2, we can calculate the equilibrium concentrations of all possible reaction products. (Reasonable estimates for the magnitudes of these constants can be obtained from various experimental data.) We will not present details of the calculations but rather the general concepts on which they are based.

The accompanying figure shows that seven complexes contain one antibody. The equilibrium concentrations of each of these complexes can be expressed as a function of K, J_1, and the $(BPO)_1$ and $(BPO)_2$ concentrations multiplied by the equilibrium concentration of free antibody. Therefore, W_1, the total equilibrium concentration of complexes containing one antibody, is obtained simply by adding together the equilibrium concentration of each of the complexes. We find that

$$W_1 = X f\left([(BPO)_1],[(BPO)_2],K,J_1\right) \quad ,$$

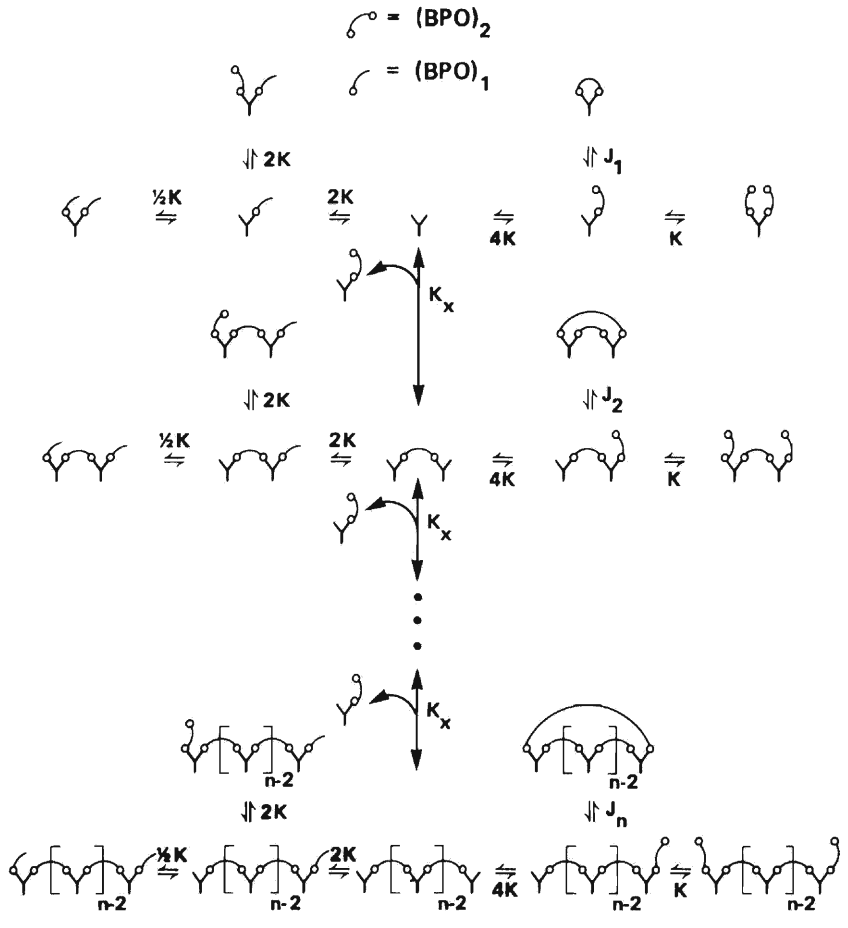
where

$$f = \left\{1 + K[(BPO)_1] + 2K[(BPO)_2]\right\}^2 + 4KJ_1[(BPO)_2] \quad .$$

In these equations $[(BPO)_1]$ and $[(BPO)_2]$ are the concentrations of $(BPO)_1$ and $(BPO)_2$, respectively, and X, the only unknown, is the equilibrium concentration of free antibody.

The accompanying figure also shows that seven complexes contain two antibodies. We can express the concentration of each of these complexes as a function of K, J_2, $[(BPO)_1]$, and $[(BPO)_2]$ multiplied by the concentration X_2 of the crosslinked chain containing two antibodies with a free binding

Sidebar



Shown here are all the binding reactions that can occur when BPO-specific IgE antibodies on basophils are exposed to the monovalent and bivalent synthetic penicillin allergens $(BPO)_1$ and $(BPO)_2$. The equilibrium constants for each reaction are also given.

site on the antibody at each end. But X_2 is in turn a function of K, $[(BPO)_2]$, and X, namely

$$X_2 = 4KK_x[(BPO)_2]X^2 \quad .$$

We can continue this process iteratively and develop a general expression for W_n, the equilibrium concentration of complexes containing n antibodies, as a function of $[(BPO)_1]$, $[(BPO)_2]$, K, K_x, J_n, and X:

$$W_n = X^n\{4KK_x[(BPO)_2]\}^{n-1}$$

$$\times f([(BPO)_1],[(BPO)_2],K,J_n) \quad .$$

The conservation law for total antibody concentration X_T leads to the equation

$$X_T = \sum_{n=1}^{\infty} nW_n \quad .$$

When we express all the W_n in this infinite series in terms of X, the infinite series can be summed, and we obtain an algebraic equation that can be solved for X. By substituting the solution for X into the expression for a particular W_n or for the equilibrium concentration of a particular complex, we can compute values for these expressions.

Because of the central role of crosslinks in the activation and desensitization of basophils, we are particularly interested in X_{poly}, the fraction of antibodies incorporated at equilibrium into complexes containing more than one antibody, that is, in the fraction of crosslinked antibodies. An expression for X_{poly} is easily derived since

$$X_{poly} \equiv \sum_{n=2}^{\infty} nW_n = (X_T - W_1)/X_T \quad .$$

Since this calculation was first presented, much theoretical work has been done on both the equilibrium and the kinetic theory of binding of multivalent antigens to antibodies on cell surfaces. As a result primarily of the work of Alan Perelson (Los Alamos), Charles DeLisi (National Institutes of Health), and Catherine Macken (Lincoln College, New Zealand), much progress has been made toward understanding the bonding of these more complicated antigens. ■

bodies is increased (by, for example, longer incubation), the $(BPO)_2$ concentration at which the maximum occurs does not change (Fig. 14a). On the other hand, increasing the concentration of $(BPO)_1$, which reduces the number of potential crosslinks, causes the maximum to shift to higher $(BPO)_2$ concentrations (Fig. 14b).

Histamine Release vs Crosslinking

How do the experimental dose response curves and the theoretical crosslinking curves compare? Figure 15 shows that the dose response curves exhibit the same properties as the crosslinking curves: the $(BPO)_2$ concentration at which the maximum occurs does not change with the number of BPO-specific antibodies on the basophil surfaces (Fig. 15a) but does change with the $(BPO)_1$ concentration (Fig. 15b). There is other agreement as well. For example, the properties of the basophil do not appear in Eq. 1; only the binding between the BPO group and the antibody is important. Therefore, we predict that basophils from different donors should exhibit maxima in their crosslinking curves at the same $(BPO)_2$ concentration. This prediction is in agreement with the observation that each of three dose response curves for basophils subjected to the same experimental treatment but from different nonallergic donors had its max-

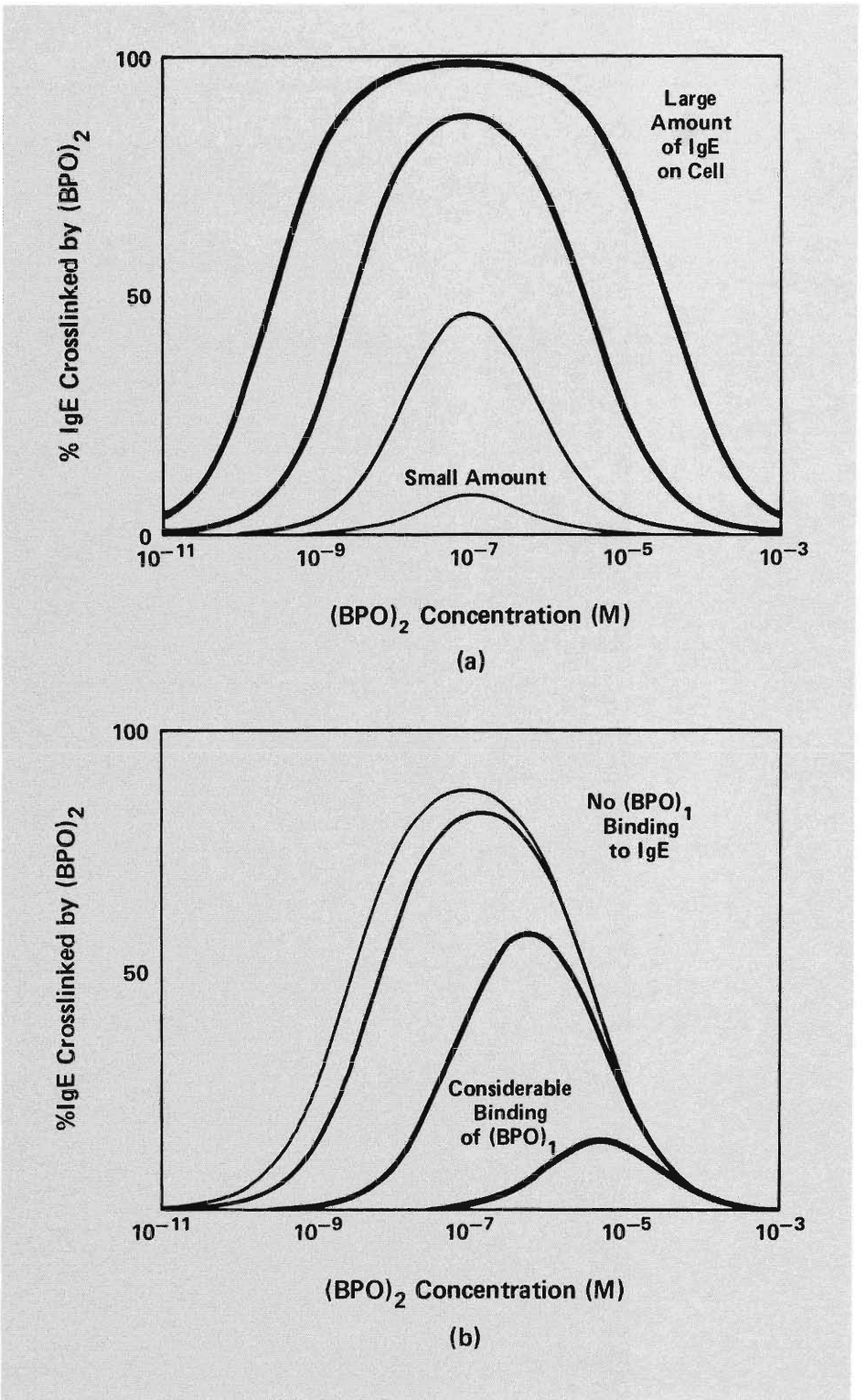
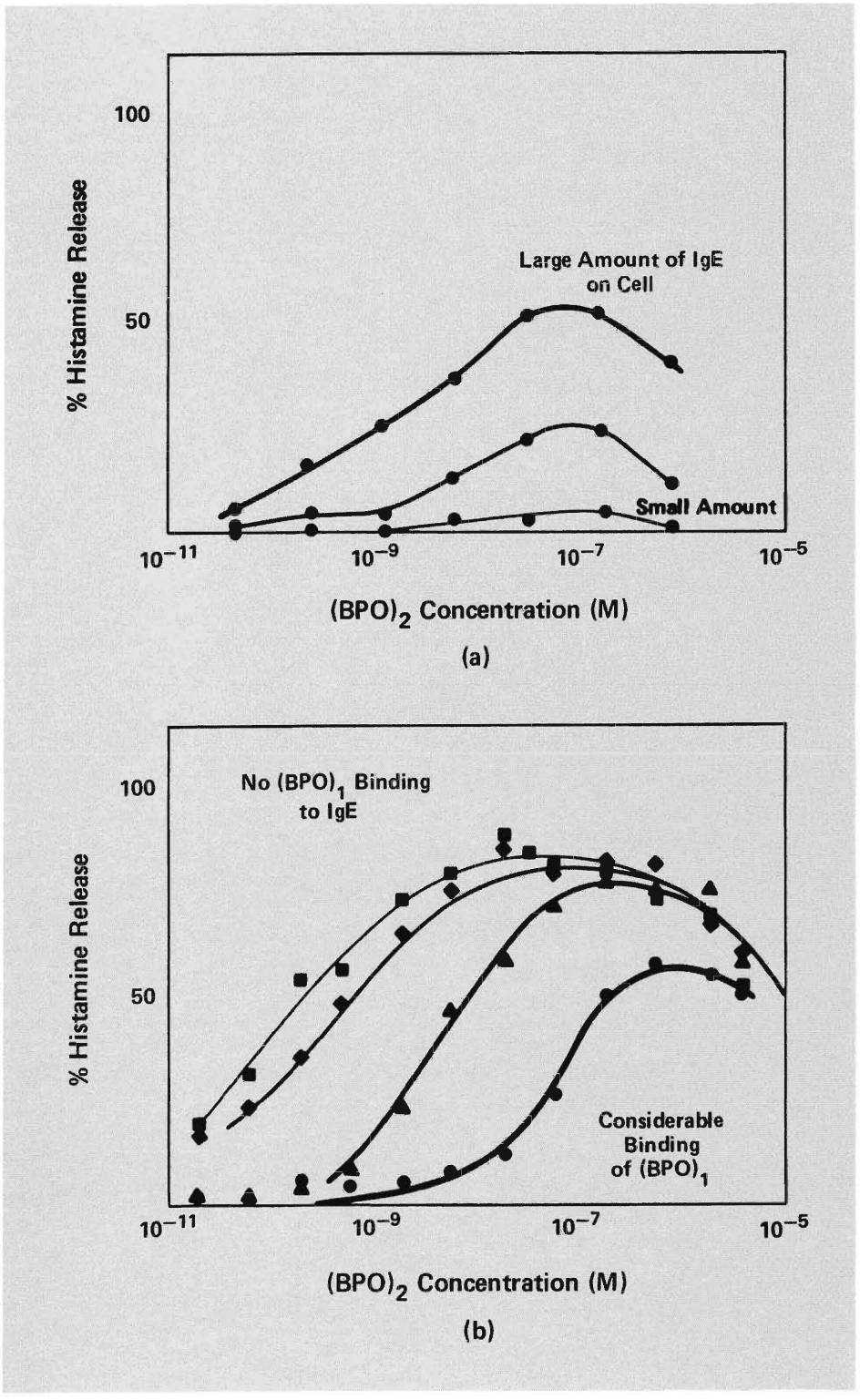


Fig. 14. Behavior of theoretical crosslinking curves. (a) As the amount of BPO-specific IgE antibodies on the basophil surfaces is increased, the crosslinking curves exhibit greater maxima, but the allergen concentration at which the maxima occur remains constant. (b) As the concentration of monovalent $(BPO)_1$ to which the BPO-specific IgE antibodies are exposed increases, the number of crosslinks decreases and the position of the maximum shifts to higher $(BPO)_2$ concentrations.



imum at the same $(BPO)_2$ concentration.

From these and other similar experiments on basophils from nonallergic donors, we concluded that the percentage of histamine released rises and falls directly with the rise and fall of the fraction of crosslinked antibodies. This behavior is quite unlike that of basophils from allergic donors. Recall that for these cells the fall in the dose response curve occurs because the number of crosslinks has increased to the point where nonspecific desensitization dominates over histamine release. In other words, too many crosslinks exist rather than too few. The difference in behavior is surely related to the fact that basophils from nonallergic donors have relatively small numbers of specific IgE antibodies bound to their surfaces, whereas cells from allergic donors have relatively large numbers.

Basophils from allergic and nonallergic donors should manifest other differences as well. In particular, can basophils from nonallergic donors be desensitized by withholding calcium ions?

Another "Off" Signal: Specific Desensitization

We found that passively sensitized

Fig. 15. Behavior of experimental dose response curves. Like the crosslinking curve shown in Fig. 12, the dose response curves for basophils from nonallergic donors exhibit maxima and are symmetric about those maxima. Further, the dependence of the dose response curves on the amount of BPO-specific antibodies on the basophil surfaces (a) and on the (BPO) concentration (b) is similar to that of the crosslinking curves (Fig. 14). The high concentration wings of the dose response curves are missing because of the difficulty in keeping high concentrations of $(BPO)_2$ in solution.

basophils from nonallergic donors do, in fact, desensitize. But they do so in a way that has not before been observed—they desensitize specifically. When exposed to $(BPO)_2$ in the absence of calcium ions, they behave in one respect just like basophils from allergic donors: they progressively lose their ability to respond to $(BPO)_2$ (by releasing histamine) when calcium ions are added. However, when exposed in the presence of calcium ions to a rabbit antibody specific to human IgE antibodies, they release histamine normally. Desensitizing with one allergen or crosslinking agent affected only the histamine release that is triggered by that allergen or crosslinking agent.

Our colleagues at Johns Hopkins performed an experiment showing that basophils from some *allergic* donors also desensitize specifically. After screening a number of individuals who were allergic to substances other than penicillin, they found one whose basophils had some fraction of their large numbers of Fc_ϵ receptors unfilled. They passively sensitized these basophils with BPO-specific IgE antibodies and then exposed them in the absence of calcium ions to $(BPO)_2$ at the concentration for maximum crosslinking of the antibodies. The cells were then exposed in the presence of calcium ions to either $(BPO)_2$, ryegrass allergen (the individual's natural allergen), or a goat antibody specific to IgE antibodies. The results (Fig. 16) show that the cells desensitized only to $(BPO)_2$. In this case, even though the number of crosslinks between BPO-specific IgE antibodies was at a maximum, the total number was too small for nonspecific desensitization to play a significant role, and another type of desensitization came into play.

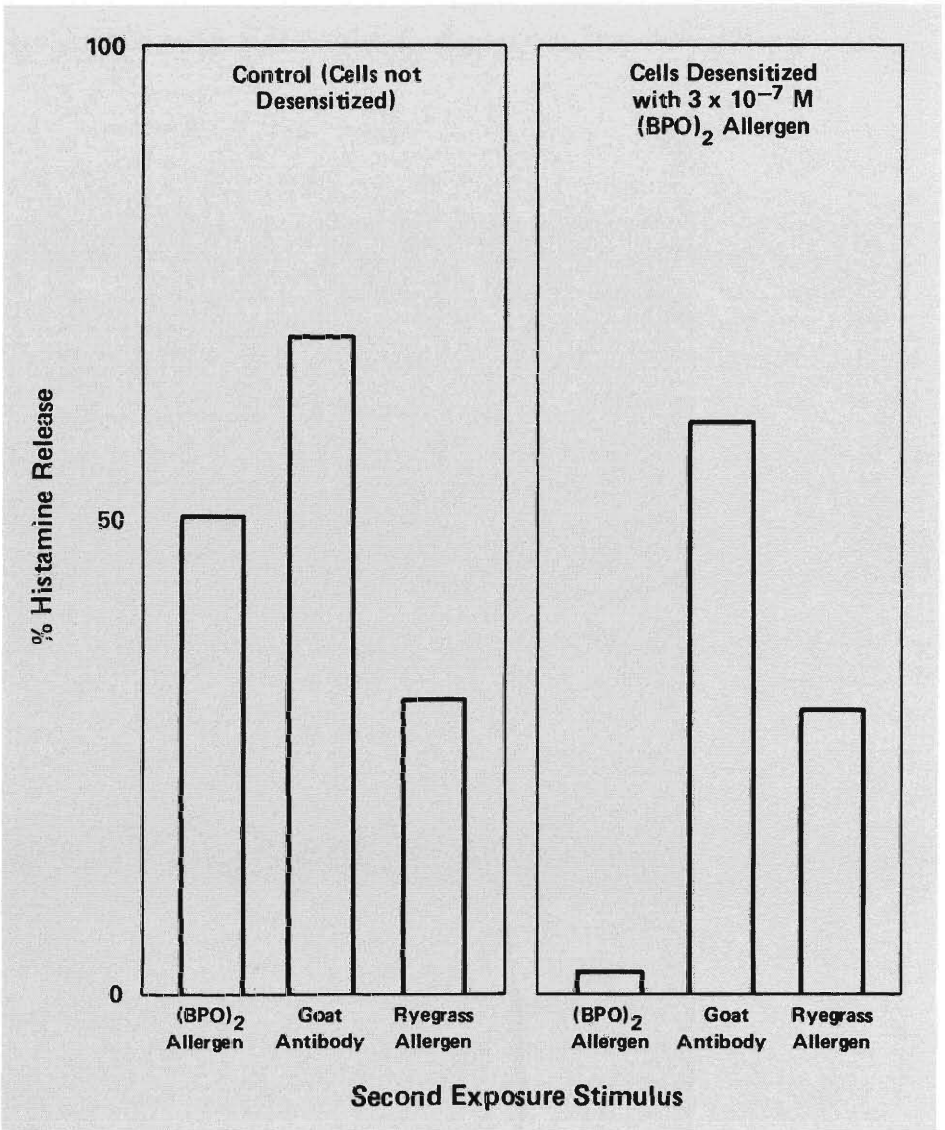


Fig. 16. Specific desensitization. The basophils studied in the experiment illustrated here were from a donor who was allergic to ryegrass. Although most of the Fc_ϵ receptors on the donor's basophils were filled with IgE antibodies specific to the ryegrass allergen, a sufficiently large number of empty Fc_ϵ receptors existed that the basophils could be passively sensitized to the BPO group. On the left are the percentages of histamine released by the passively sensitized basophils when exposed to either the synthetic $(BPO)_2$ allergen, a goat antibody specific to human IgE antibodies, or the ryegrass allergen. On the right are the percentages of histamine released by these cells when exposed to the allergens or the goat antibody after ***being desensitized to $(BPO)_2$. Only the histamine release triggered by the desensitizing agent is significantly affected.***

In a subsequent study we found that allergic individuals whose basophils desensitize specifically have much lower concentrations of IgE antibodies in their serum than do allergic individuals whose basophils desensitize nonspecifically. This fact is further support for the suggestion that specific desensitization is a phenomenon associated with small numbers of specific IgE antibodies on the basophil surfaces.

Because $(BPO)_1$, which can bind but not crosslink IgE antibodies, does not desensitize basophils, we know that specific desensitization is also triggered by crosslinks. We therefore predict that basophils from nonallergic donors will undergo the greatest specific desensitization at the allergen concentration producing the maximum number of crosslinks, that is, the concentration at which histamine release is a maximum. To test this prediction we performed the following experiment. Again, basophils from nonallergic donors were passively sensitized to $(BPO)_2$. The dose response curve for these

TABLE I

CHARACTERISTICS OF SPECIFIC AND NONSPECIFIC DESENSITIZATION

	Specific Desensitization	Nonspecific Desensitization
Substances to which the basophils desensitize	Only the desensitizing allergen	All allergens and crosslinking agents
Relation to number of specific IgE antibodies on the basophil surfaces	Occurs when the number is small	Occurs when the number is large
Relation to number of crosslinks on the basophil surfaces	Is maximum when the number, although small, is maximum	Is maximum when the number is maximum
Allergen concentration at which maximum histamine release occurs	Identical to concentration at which crosslinks and desensitization are maximum	Lower than the concentration at which crosslinks and desensitization are maximum
Explanation in model	Linked Fc_ϵ receptors are altered	Limited amount of gating factor is available

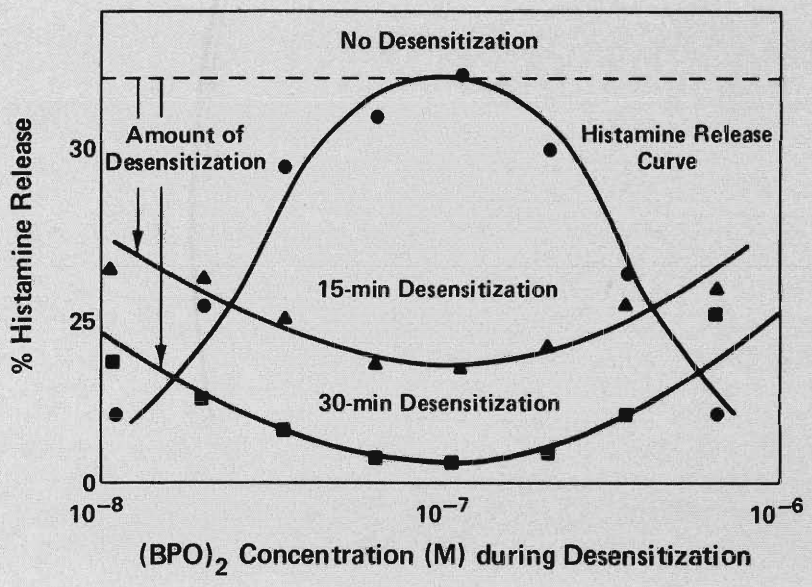


Fig. 17. The basophils studied in the experiment illustrated here were from a nonallergic donor and had been passively sensitized to the BPO group. The dose response curve for these cells exhibited a maximum at a $(BPO)_2$ concentration of 10^{-7} molar. The cells were then specifically desensitized to $(BPO)_2$ by exposing them in the absence of calcium ions to various $(BPO)_2$ concentrations for either 15 or 30 minutes. Finally, the percentages of histamine released by the cells when exposed in the presence of calcium ions to a $(BPO)_2$ concentration of 10^{-7} molar was measured. The difference between the maximum histamine released (dashed line) and the measured histamine release (lower curves) is a measure of the amount of specific desensitization undergone by the cells at that (BPO) concentration. Note that the desensitization is greatest for a desensitizing $(BPO)_2$ concentration of 10^{-7} molar, the $(BPO)_2$ concentration for maximum histamine release.

cells exhibited a maximum at a $(BPO)_2$ concentration of 10^{-7} molar. Groups of the sensitized cells were then desensitized by exposing them for a fixed time to $(BPO)_2$ in the absence of calcium ions. The $(BPO)_2$ concentration was varied from group to group. Finally, the desensitized cells were exposed in the presence of calcium ions to $(BPO)_2$ at the concentration for maximum histamine release, or 10^{-7} molar. If no desensitization had taken place, all the groups of cells would have released the maximum amount of histamine. But desensitization did, in fact, occur and was evidenced by the cells' release of less than the maximum amount of histamine. Further, the group of cells desensitized at a $(BPO)_2$ concentration of 10^{-7} molar released the least amount of histamine and thus underwent the greatest amount of desensitization (Fig. 17).

Recently, in collaboration with Henry Metzger of the National Institutes of Health, we used covalently linked pairs of IgE antibodies to show that large aggregates of crosslinked IgE antibodies are not required to induce specific desensitization. Just as for histamine release, the formation of linked pairs of IgE antibodies is the "unit" signal for specific desensitization.

The two modes of desensitization are compared in Table I. Are they related or are they independent mechanisms?

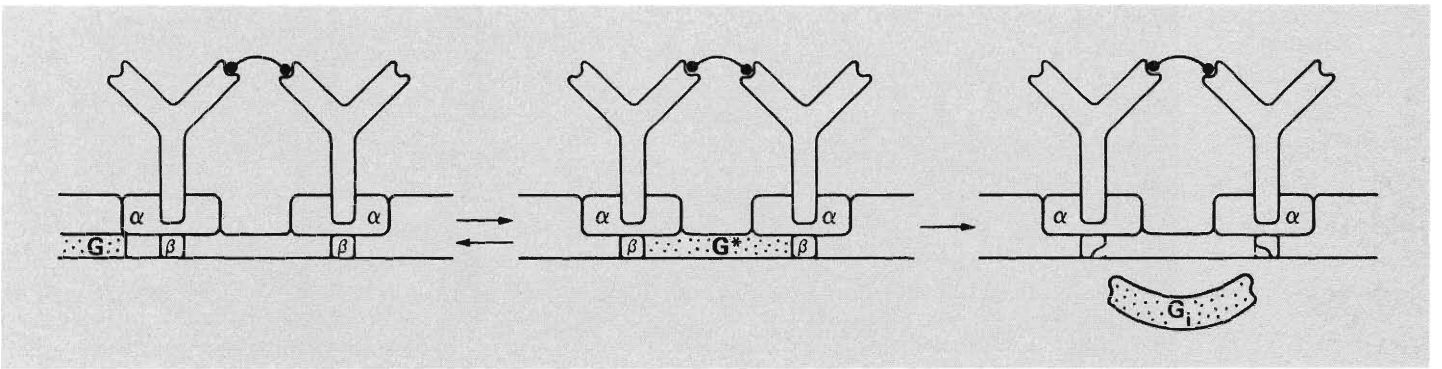


Fig. 18. Desensitization model. In this model an open calcium channel (G) is formed when an allergen crosslinks two IgE antibodies and a gating factor (G) combines with a part (β) of the Fc_ϵ receptors to which the antibodies are bound. Decay of the channel results in loss of the gating factor (G_i), which can account for nonspecific desensitization, and alterations in the β portion of the receptors, which can account for specific desensitization.*

The Transition from Specific to Nonspecific Desensitization

We have evidence that basophils with relatively small numbers of specific IgE antibodies on their surfaces desensitize specifically and that basophils with relatively large numbers of specific IgE antibodies on their surfaces desensitize nonspecifically. Perhaps if the number of specific IgE antibodies on the basophil surfaces could be varied over a large enough range, the type of desensitization exhibited by the basophils would undergo a smooth transition from specific through partially nonspecific to completely nonspecific.

In 1981 Donald MacGlashan and Lawrence Lichtenstein performed an experiment to test this suggestion. A major difficulty they faced was finding a donor whose basophils had enough free Fc_ϵ receptors, but eventually one was located whose basophils had close to 20,000 per cell. To take full advantage of the free sites available, they very carefully purified a BPO-specific antibody preparation before passively sensitizing the basophils. They estimated that in their experiments the number of BPO-specific IgE antibodies per basophil was varied from approximately 800 to 14,000. Over this range they observed a smooth transition in the type of desensitization from specific to nonspecific, although the nonspecific desensitization was not complete. Our expectation is that if more BPO-specific IgE antibodies could be placed on the basophils, complete nonspecific desensitization would be achieved.

A Speculative Model for Desensitization

Crosslinking of IgE antibodies on the surface of a basophil causes a flow of calcium ions into the cell that triggers the release of histamine-containing granules from the cell. But crosslinking also leads to desensitization of a type determined by the number of IgE antibodies on the basophil surface.

How does this all come about? We're not certain, but we have some ideas that we have formalized in a mathematical model. The model can explain what has been observed so far and can also make predictions that can be tested experimentally. The basic features of the model are sketched in Fig. 18. We propose that when two Fc_ϵ receptors are brought into proximity by crosslinked IgE antibodies, they combine with a "calcium gating factor" in the cell membrane. This reaction forms a channel through which calcium ions flow into the basophil. We have shown experimentally that channels formed by crosslinked IgE antibodies are short-lived. We have incorporated this observation into our model by assuming that a channel rapidly decays to an inactive form. We also assume that only a limited amount of gating factor is available.

This model explains nonspecific desensitization as follows. As basophils with large numbers of IgE antibodies specific to a particular allergen are exposed to the allergen in the absence of calcium ions, so many calcium channels are formed that the supply of gating factor is exhausted. When later exposed to *any* allergen in the presence of calcium ions, no further calcium channels can be formed and those formed during desensitization have decayed. Hence, no histamine is released.

Calcium ions act as stimulatory signals not only for basophils and mast cells but also for a variety of other cells, some of which undergo processes similar to desensitization. For example, cells in the blowfly's salivary gland can be stimulated to secrete by an influx of calcium ions triggered by the molecule 5-hydroxytryptamine. Continued

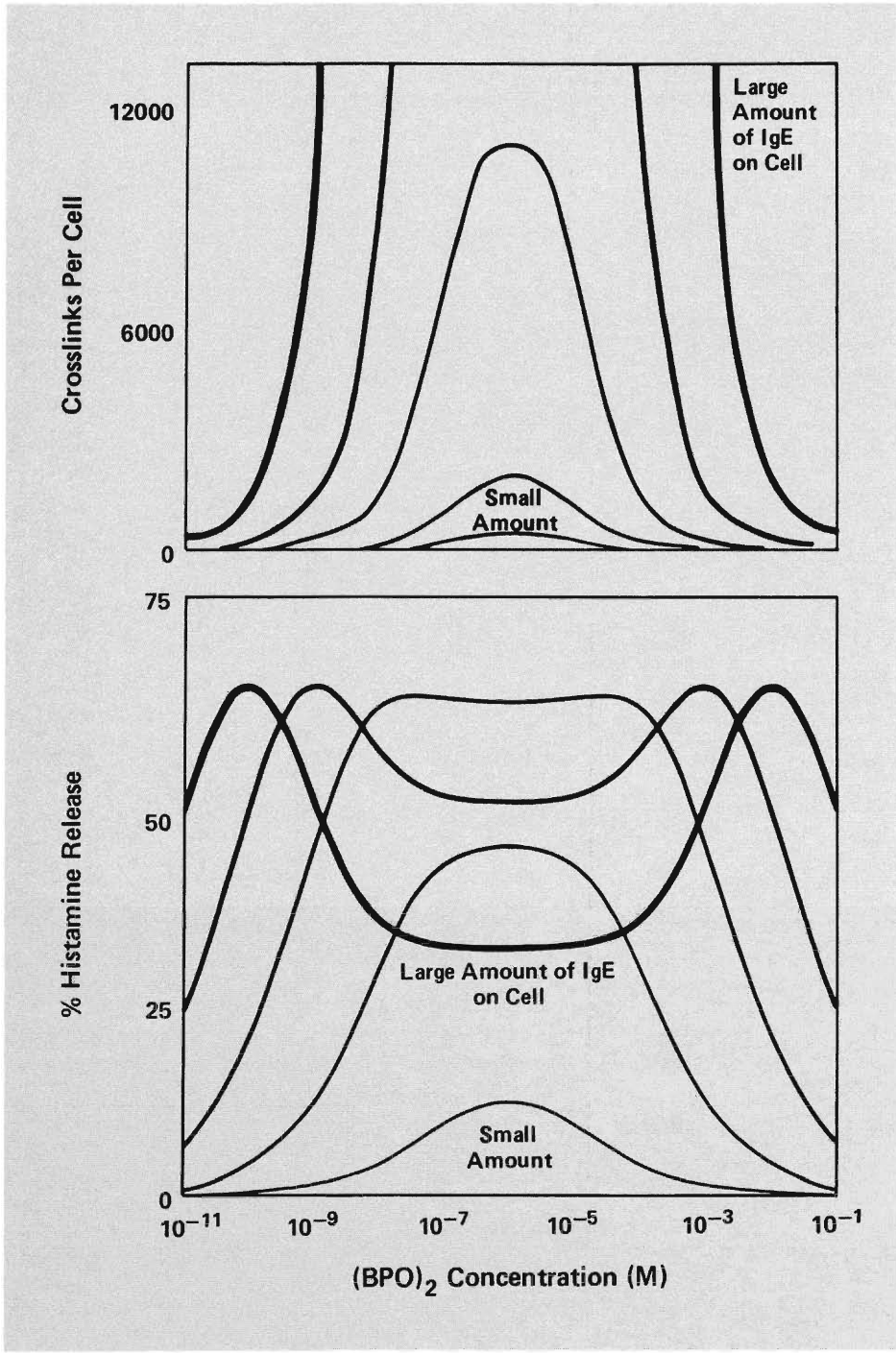


Fig. 19. These two graphs illustrate the relationship predicted by the model of Fig. 18 between crosslinks and histamine release. The upper graph shows that very large numbers of crosslinks can form on basophils with large numbers of BPO-specific IgE antibodies on their surfaces. These large numbers of crosslinks are accompanied, at $(BPO)_2$ concentrations around the optimum for release, by reductions in the percentage of histamine released (lower graph). This reduction represents the transition from specific to nonspecific desensitization.

treatment with 5-hydroxytryptamine leads to a shutdown of the mechanism for calcium ion transport and, hence, to a shutdown of secretion. The shutdown occurs because the calcium gating factor, which has been identified in this case as phosphatidylinositol, is only a minor constituent of the cell membranes and is eventually used up. When phosphatidylinositol is restored to the cells, desensitization is reversed, and the cells once again secrete normally.

What the calcium gating factor is in human basophils, if there is one, is not known. We have attempted to identify a gating factor by incorporating phosphatidylinositol and other likely candidates into nonspecifically desensitized basophils, but none of these substances caused their release of histamine to revert to normal.

Another feature of the model is invoked to explain specific desensitization. We propose that decay of a calcium channel is accompanied by inactivation of the Fc_ϵ receptors between which the calcium channel was formed. This inactivation affects the course of events as follows. As basophils with small numbers of IgE antibodies specific to a particular allergen are exposed to that allergen in the absence of calcium ions, only a small number of calcium channels are formed and the supply of gating factor is not exhausted. But as the calcium channels decay, so also do the Fc_ϵ receptors filled with these crosslinked antibodies. This inactivation of the receptors in effect inactivates all the IgE antibodies specific to the desensitizing allergen, and later exposure to that allergen in the presence of calcium ions does not cause histamine release. On the other hand, later exposure in the presence of calcium ions to a different allergen can cause histamine release because that allergen crosslinks IgE antibodies specific to itself. These crosslinked antibodies can then combine with the remaining gating factor to form calcium channels.

The transition between the two types of desensitization occurs when the number of crosslinks becomes significant compared to the amount of gating factor. These ideas also are summarized in Table I. Figure 19 shows the relationship between crosslinks and histamine release predicted by the model. The lower three curves in both graphs essentially duplicate the crosslinking and dose response curves for basophils with limited numbers of specific IgE antibodies on their surfaces (Figs. 14a and 15a). The upper two curves in both graphs show the effect of large numbers of specific IgE antibodies. In particular, the

onset of nonspecific desensitization is indicated at the middle $(BPO)_2$ concentrations by the continued rise (off scale) in the number of crosslinks accompanied by a decrease in the percentage of histamine released.

Our model couples specific and nonspecific desensitization. Because of this coupling the model makes strong predictions about the relationship between the time course of specific and nonspecific desensitization for experiments carried out with cells from the same donor. It also makes predictions about what happens when basophils desensitized with one allergen are later exposed to the same or different allergens. Experiments to test these predictions are in progress at Johns Hopkins.

Of course it could be that specific and nonspecific desensitization are not coupled at all. Indeed, our first guess was that nonspecific desensitization came about because some gating factor was used up whereas specific desensitization was the result of a totally independent process called receptor-mediated endocytosis. This process would literally transfer the allergen-linked IgE antibodies from the cell surface to the cell interior. This guess was based on a number of examples of endocytosis in which cells internalize a variety of their own surface receptors. In some cases the internalization is triggered by the binding of a molecule to the receptor and in other cases by the crosslinking of molecules bound to the receptors. However, Donald MacGlashan has recently shown that endocytosis does not occur in specifically desensitized basophils. He passively sensitized basophils with BPO-specific IgE antibodies and specifically desensitized these cells by exposing them to $(BPO)_2$ in the absence of calcium. He then washed off the $(BPO)_2$ and exposed the cells to radioactively labeled molecules containing many BPO groups. He observed that the basophils specifically bound the radioactive label. Therefore, the BPO-specific IgE antibodies still remained on the basophil surfaces after specific desensitization.

Conclusion

Allergic reactions of the immediate type arise because of a complicated chain of events: exposure to an allergen, recognition of the allergen by the immune system, production of IgE antibodies, sensitization of basophils or mast cells, re-exposure to the allergen, triggering of basophils or mast cells to release histamine and other chemicals, and response of the body to those chemicals. Treatments of allergies are designed to break this chain. Of course, the best thing to do is to avoid the allergen. This is straightforward if you are allergic to cod fish, but impossible if you are allergic to juniper pollen and insist on living in New Mexico.

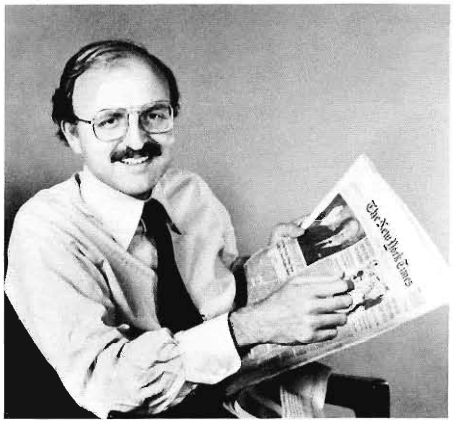
If you can't stay away from the allergen, you can try to break the chain by manipulating the immune response. Almost all allergy "shots" are directed toward this end; they attempt to affect, not the basophils or the mast cells, but the cells of the immune system that produce the antibodies. Exposures to low concentrations of an allergen (the shots) over long periods can sometimes desensitize these cells. The cells are then said to be tolerized because they no longer produce antibodies that bind to the allergen but rather tolerate its presence. If tolerance can be maintained so that the IgE response is constantly blocked, the chain is broken and the patient is free of symptoms. Unfortunately, a substantial fraction of those treated with low doses of allergen do not become tolerant to the allergen.

A second type of treatment involves increasing the immune response rather than decreasing it. The idea is to produce in an allergic individual such high concentrations of antibodies of classes other than IgE that these antibodies, flowing in the blood, bind to the allergen and prevent it from triggering basophils and mast cells. This raising of the "blocking" antibody concentration has been highly successful in the treatment of bee sting allergy. Allergic individuals receive regular injections of pure bee venom at concentrations that raise and maintain a high IgG response but do not trigger histamine release from basophils. If such an individual is stung by a bee, the IgG antibodies in solution bind to the bee venom and prevent it from crosslinking the venom-specific IgE antibodies on basophils. This treatment, however, is much less successful for allergens that are inhaled than for allergens that are injected.

An alternative to immunotherapy is drug therapy. Drugs such as the antihistamines have been used for some time in attempts to prevent the body from responding to the chemicals released during allergic reactions. Some of these drugs also inhibit, at least to some extent, the release of chemicals from the basophil and mast cell granules.

These are the major approaches to the treatment of immediate hypersensitivity. At the moment there is no therapy in use that is designed to bring about basophil and mast cell desensitization. Some cases have been reported in which immunotherapy produced desensitization of basophils, but this result was fortuitous rather than by design. Perhaps our expanding grasp of the desensitization process will alter this situation. ■

AUTHORS



Byron Goldstein was born and grew up in New York City. He received his Bachelor of Science in physics from the City College of New York in 1961 and his Ph.D. in theoretical physics from New York University in 1967. He began to work seriously on problems related to biology while he was a National Institutes of Health postdoctoral fellow at the University of California, San Diego. He joined the Theoretical Biology and Biophysics Group at the Laboratory in 1975. Before coming to Los Alamos he was a Professor of Physics and member of the Biophysics Group at Fairleigh Dickinson University.

Micah Dembo earned his Bachelor of Science in mathematics from Allegheny College in 1972 and his Ph.D. in biomathematics from Cornell University Medical College in 1977. After finishing graduate work, he came to Los Alamos as a postdoctoral fellow in the Theoretical Biology and Biophysics Group and remained as a staff member after the postdoctoral appointment ended. During his years in the group he has worked on a number of theoretical problems of importance in biology. In addition to developing mathematical models of cell activation and desensitization, he has worked on the modeling of cooperative interactions in proteins, on diffusion reaction problems, particularly with regard to membrane transport phenomena, and on fluid mechanical models of cell motility.

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